

PATENT  
Docket No. 204372000320

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on November 4, 1996.

Nancy J. Robins  
NANCY J. ROBINS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#9  
P.D.

In the application of:

Lynn E. Spitler et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

**DECLARATION OF LYNN E. SPITLER  
PURSUANT TO 37 C.F.R § 1.132**

Box AF  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, Lynn E. Spitler, declare as follows:

1. I am a coinventor in regard to the above-referenced patent application, and have been supervising clinical trials using antitumor vaccines which contain recombinant human prostate-specific antigen (PSA) as the active ingredient. I am an experienced immunologist and medical doctor. A copy of my *curriculum vitae* is attached hereto as Exhibit A.

2. I note that The Examiner makes the point several times that previous attempts to actively immunize patients with prostate adenocarcinoma cells admixed with adjuvant have shown little or no therapeutic benefit. However, the use of whole tumor cells is not analogous to the use of recombinant protein such as purified PSA. Whole PSA is not represented on the surface of the

tumor cells; thus, the patients would not be expected to be effectively immunized to PSA via this approach. PSA is synthesized within the tumor cells and secreted; therefore, the patients' immune system might be exposed to small amounts of PSA through this approach as some of the tumor cells die and release the internal PSA; these small amounts of antigen would be presented to the immune system in the context of all the other antigens present on and in the tumor cells. This would not be likely to result in an immune response to the PSA. Peptides derived from PSA are present on the surface of the tumor cells, presented in the context of HLA molecules. For these to induce an immune response, it would be expected that they would have to be taken up by the professional antigen presenting cells and represented on the surface of these cells. Again, this would be occurring in the presence of all the other antigens present on and in the tumor cells.

3. Thus, one cannot take failure of the approaches using whole tumor cells to indicate that immunization with specific antigens will fail (including antigens overrepresented in the prostate gland, an immunologically effective portion thereof, or an antiidiotypic antibody). Indeed, it is the recognition that the use of pure antigens may represent a more effective means of immunization for cancer therapy which has led to intense activity in this field and numerous clinical trials (Spitler, L.E., Engineered Vaccines for Cancer, *Sixth International Congress on Anti-Cancer Treatment* (1995) Paris, February 6-9, 1996; Spitler, L.E., Cancer Vaccines: The Interferon Analogy, *Cancer Biotherapy* (1995) 10:1-3 (copies attached).

4. Clinical trials in a number of patients have been initiated using recombinantly produced human PSA. PSA is a well known glycoprotein with a molecular weight of 33-34 kDa. PSA was cloned, expressed, and produced by large scale suspension cultures of High Five™ insect cells infected with recombinant PSA-baculovirus. PSA has the amino acid sequence:

D L I V G G W E C E K H S Q P W Q V L V  
A S R G R A V C G G V L V H P Q W V L T  
A A H C I R N K S V I L L G R H S L F H  
P E D T G Q V F Q V S H S F P H P L Y D

M S L L K N R F L R P G D D S S H D L M  
 L L R L S E P A E L T D A V K V M D L P  
 T Q E P A L G T T C Y A S G W G S I E P  
 E E F L T P K K L Q C V D L H V I S N D  
 V C A Q V H P Q K V T K F M L C A G R W  
 T G G K S T C S G D S G G P L V C N G V  
 L Q G I T S W G S E Q C A L P E R P S L  
 Y T K V V H Y R K W I K D T I V A N P

PSA was purified from the culture supernatants by affinity chromatography using a monoclonal antibody specific to PSA and incorporated into liposomes of the following composition:

Each ml. (one dose) contained:

Component	Quantity (mg/ml)
Prostate Specific Antigen	Approximately 0.10
Monophosphoryl Lipid A	0.20
Dimyristoyl phosphatidylcholine	61.01
Dimyristoyl phosphatidylglycerol	6.89
Cholesterol	29.00
Polysorbate 80	0.10*

Buffer: 20 mM TRIS-glycine in 140 mM NaCl

\*Maximum quantity that can be incorporated. The actual amount incorporated is unknown.

5. - Six (6) patients were immunized with the prostate cancer vaccine described above. Each patient was given 1 ml of the vaccine intramuscularly, divided into 2 sites, on days 0, 30, and 60. An additional two (2) patients have been treated by intravenous administration of the product with the same dose and schedule of administration. All patients were carefully monitored for adverse effects through clinical and laboratory evaluation. No adverse event attributable to the vaccine was observed in any patient. Specifically, there were no adverse events suggesting an autoimmune reaction to cross-reacting antigens.

6. Immunologic tests of T and B cell responses were performed before each immunization and 2 weeks after each immunization. Evidence of T-cell immune responses was observed. (Harris, D.T., et al., Active Specific Immunization of Patients with Hormone-refractory Prostate Cancer using OncoVax-P™, ASCO Proceedings (1996)) (copy enclosed).

7. For immunologic testing of patients, a pool of peptides representing CTL epitopes of PSA was used:

Amino Acid Numbers	Sequence
29-37	VLVHPQWVL
98-106	MLLRLSEPA
141-150	FLTPKKLQCV
146-154	KLQCVDLHV
154-163	VISNDVCAQV

Peripheral blood mononuclear cells were harvested at the times indicated and incubated with the PSA peptide pool. On the third day of culture, Interleukin-2 (IL-2) was added. On day 7, the cultures were restimulated with autologous antigen presenting cells and the PSA peptide pool. The cultures were assayed on day 19 to determine the levels of gamma interferon and Interleukin-4 (IL-4) production. Results in the first four patients studied showed an increase in the production of these cytokines in some of the samples after immunization, as compared to before immunization, thus indicating a T-cell response. These results are shown in Exhibit B.



8. The foregoing results show that in clinical trials, the vaccine of the invention causes no adverse side effects sufficient to undermine its efficacy and that the vaccine is capable of eliciting an immune response to the PSA antigen mediated by T-cells.

9. In more detail, in regard to safety, there were no local reactions at the injection site, no symptoms of prostatitis, no signs of autoimmune disease, no malaise or fevers, and no signs of allergic reactions.

10. All of the patients had metastatic disease, had failed hormonal therapy, and had rising levels of PSA at the time of entry into the study.

11. As shown in the table below, and in Exhibit B, two of the six patients (patients no. 2 and no. 3) had immunological responses to PSA and three others had some suggestion of reaction (patients no. 1, no. 4 and no. 5). Lymphocytes from patient no. 2 showed proliferation to PSA and to PSA peptides as well as production of the cytokines  $\gamma$ -interferon and interleukin-4 in response to PSA peptides. The lymphocytes from patient no. 3 showed proliferation in response to PSA in two separate tests and this patient had a positive skin response to PSA. We were not able to measure CTLs directly because the assay is still under development; however, the cytokine production in response to PSA peptide stimulation shown in two patients is correlated with CTL development. The following table summarizes the results obtained. N.T. refers to not tested.

Immologic Responses Summary							
Patient #	PSA Skin Test	Lympho #1 PSA	Lympho #2 PSA	Lympho Peptide	Cytokine Gamma IFN	Cytokine IL-4	Antibody
1. AW	-	-	-	+/-	-	+	0
2. JH	N.T.	+	-	+	+	+	0
3. MD	+	+	+	-	-	+/-	+/-
4. MED	-	N.T.	-	-	+/-	-	0
5. HN	-	N.T.	+/-	-	N.T.	N.T.	0
6. JLB	-	N.T.	-	-	N.T.	N.T.	0

12. Exhibit C contains copies of overhead transparencies prepared for formal presentation of results of the clinical study.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Tiburon, California on

11/1/96  
Date

Lynn E Spiller  
Lynn E. Spiller

## CURRICULUM VITAE

LYNN E. SPITLER, M.D.

1895 Mountain View Drive  
Tiburon, California 94920

(415) 435-9861  
FAX (415) 435-6851

Social Security #366-38-1697

### Education:

University of Michigan, Ann Arbor, Michigan	1956-1959
University of Michigan Medical School Ann Arbor, Michigan M.D. (cum laude)	1959-1963

### Training:

Intern	Highland Alameda County Hospital Oakland, California	1963-1964
Resident	University of California School of Medicine San Francisco, California	1964-1966
Research Fellow	H.S. Lawrence, M.D. Department of Medicine - Immunology New York University New York, New York	1966-1967
Immunology Trainee	H. Hugh Fudenberg, M.D. University of California School of Medicine San Francisco, California	1967-1969

### Teaching Appointments and Employment:

Instructor of Medicine in Residence University of California School of Medicine San Francisco, California 94143	1970-1971
Assistant Professor of Medicine in Residence University of California School of Medicine San Francisco, California 94143	1971-present
Research Associate Cancer Research Institute University of California School of Medicine San Francisco, CA 94143	1971-1978
Director, Melanoma Center Northern California Health Center San Francisco, California 94118	1978-1990
Director of Research Children's Hospital of San Francisco San Francisco, California 94118	1978-1981
Member, Graduate Group in Comparative Pathology Department of Comparative Pathology University of California, Davis Davis, California	1976-1981
Senior Vice President XOMA Corporation 2910 Seventh Street Berkeley, California 94710	1981-1988
Associate Scientific Director Biotherapeutics, Inc. 357 Riverside Drive Franklin, Tennessee, 37065-1676	1988-1989

Director  
Northern California Melanoma Centers  
1895 Mountain View Drive  
Tiburon, California 94920

1990-present

President  
Jenner Technologies  
1895 Mountain View Drive  
Tiburon, California 94920

1991-present

**Awards and Honors:**

Recipient: Dernham Senior Fellowship  
California Div. of the American Cancer Society

1969-1971

Recipient: Research Career Development Award  
National Institutes of Health

1971-1976

Alpha Omega Alpha (Junior year)

1961

Outstanding Young Women of America

1968

Who's Who of American Women

1972

Who's Who in the West

1973

**Board Certification:**

American Board of Internal Medicine  
American Board of Allergy and Immunology

1972

1974

**Licensure:**

Michigan 25985  
New York 96454  
California C-26446

**Memberships in Professional Societies:**

American Association of Immunologists  
American Association for the Advancement of Science  
Alpha Epsilon Iota  
Western Society for Clinical Research  
American Federation of Clinical Research  
Society of Biological Therapy  
American Association for Cancer Research

**Patents:**

Patent #4,489,810 for "Composition and Method for Transplantation Therapy"  
Patent #4,590,071 for "Human Melanoma Specific Immunotoxins"

**PUBLIC SERVICE**

**National Review Committees:**

Allergy and Immunology Research Committee, NIAID, NIH	1976-1980
Merit Review Board in Immunology, VA, Washington, D.C. (Chairman 1979-1980)	1976-1980

**Editorial Boards:**

The Journal of Immunology	1975-1978
The International Journal of Immunopharmacology	1979-1984
Immunologia Clinica e Sperimentale	1982-1986
Antibody Immunoconjugates and Radiopharmaceuticals (Associate Editor)	1987-present
Molecular Biotherapy	1987-present
Cancer Biotherapy	1991-present

**Manuscript Reviews:**

American Review of Respiratory Disease  
Annals of Allergy  
Annals of Internal Medicine  
Archives of Dermatology  
Archives of Internal Medicine  
California Medicine  
Cancer  
Cancer Immunology and Immunopathology  
Cancer Research  
Cellular Immunology  
Chest  
Infection and Immunity  
Infectious Disease and Immunology  
International Journal of Immunopharmacology  
Journal of Clinical Investigation  
Journal of Immunology  
Journal of Infectious Diseases  
Journal of the American Academy of Dermatology  
Molecular Biotherapy  
Nature  
New England Journal of Medicine  
Science  
The Western Journal of Medicine

**Special Consultant:**

National Institutes of Health Grant Reviews  
National Institutes of Health Site Visits  
United States Tuberculosis Panel Task Group  
Atomic Energy Commission Site Visits  
Public Education Panel, National Multiple Sclerosis Society  
Review of Grant Applications for the National Science Foundation  
Enterprise for High School Students, Medical Apprenticeship Program,  
San Francisco, California  
Board of Directors, San Francisco Unit, American Cancer Society  
Research and Human Experimentation Committee, Children's Hospital  
of San Francisco  
U.S. Energy Research Development Administration Site Visits

## BIBLIOGRAPHY

(Excluding Abstracts)

LYNN E. SPITLER, M.D.

1. Spitler L.E., Cochrum K.C., and Fudenberg H.H.: Mycoplasma inhibition of phytohemagglutinin stimulation of lymphocytes. Science 161:1148-1149, 1968.
2. Spitler L.E., Huber H., and Fudenberg H.H.: Inhibition of capillary migration by antigen-antibody complexes. J. Immunol. 102:404-411, 1969.
3. Cochrum K.D., Spitler L.E., Najarian J.S., and Fudenberg H.H.: A new source of large numbers of lymphocytes and studies on their culture. In Proceedings of the Third Annual Leukocyte Culture Conference. Edited by W.O. Rieke, Appleton-Century-Crofts, New York, New York, 1969, pp. 169-175.
4. Spitler L.E., and Lawrence H.S.: Studies of lymphocyte culture; products of sensitive lymphocyte-antigen interaction. J. Immunol. 103:1072-1077, 1969.
5. Spitler L.E., Benjamini E., Young J.D., Kaplan H., and Fudenberg H. H.: Studies on the immune response to a characterized antigenic determinant of the tobacco mosaic virus protein. J. Exp. Med. 131:133-148, 1970.
6. Spitler L.E., and Fudenberg H.H.: Multiple antibody response following busulfan therapy. Vox Sang. 18:450-458, 1970.
7. Spitler L.E., and Fudenberg H.H.: Products of interaction of antigen-sensitive leukocytes and antigen: further characterization of the mitogenic factor. J. Immunol. 104:544-549, 1970.
8. Levin A.S., Spitler L.E., Stites D.P., and Fudenberg H.H.: Wiskott-Aldrich syndrome: a genetically determined cellular immunological deficiency. Clinical and laboratory responses to therapy with transfer factor. Proc. Natl. Acad. Sci. 67:821-827, 1970.
9. Spitler L.E., Keuppers F., and Fudenberg H.H.: Normal macrophage function in pulmonary alveolar proteinosis. Am. Rev. Resp. Dis. 102:975-977, 1970.



10. Spidler L.E., Levin A.S., Huber H., and Fudenberg H.H.: Prediction of results of transfer factor therapy in the Wiskott-Aldrich syndrome by monocyte IgG receptors. In Proceedings of the Sixth Leukocyte Culture Conference. Edited by M.R. Schwartz. Academic Press, Inc., New York and London, 1972, pp. 795-803.
11. Henderson W.R., Fukuyama K., Epstein W.L., and Spidler L.E.: In vitro demonstration of delayed hypersensitivity in patients with berylliosis. J. Invest. Dermatol. 58:5-8, 1972.
12. Senyk G., Nitecki D.E., Spidler L.E., and Goodman J.W.: The immune response to glucagon in conjugated form. Immunochemistry 9:79-110, 1972.
13. Henderson W.R., Fukuyama K., Epstein W.L., and Spidler L.E.: Demonstration of cellular immunity to tumor-specific antigens of malignant melanoma in hamsters by inhibition of macrophage migration. J. Invest. Dermatol. 58:299-332, 1972.
14. Spidler L.E., Von Muller C., Fudenberg H.H., and Eylar E.A.: Experimental allergic encephalitis: dissociation of cellular immunity to brain protein and disease production. J. Exp. Med. 136:156-174., 1972.
15. Fudenberg H.H., Spidler L.E., and Levin A.S.: Treatment of immune deficiency. Am. J. Pathol. 69:529-535, 1972.
16. Hitzig W.H., Font Anellaz H.P., Muntener U., Paul S., Spidler L.E., and Fudenberg H.H.: Transfer factor: immunologische Grundlagen und therapeutische Erfahrungen. Schweizerische Medizinische Wochenschrift 102:1237, 1972.
17. Spidler L.E., Levin A.S., Stites D.P., Fudenberg H.H., Pirofsky B., August C.S., Steihm E.R., Hitzig W.H., and Gatt R.A.: The Wiskott-Aldrich syndrome: results of transfer factor therapy. J. Clin. Invest. 51:3216-3224, 1972.
18. Lieber E., Hsu L., Spidler L.E., and Fudenberg H.H.: Cytogenetic findings in a parent of a patient with Faconi's anemia. Clin. Genet. 3:357-363, 1972.
19. Spidler L.E., Levin A.S., and Fudenberg H.H.: Human lymphocyte transfer factor. In Methods in Cancer Research, vol. 8. Edited by H. Busch, Academic Press, New York, 1973, pp. 59-106.
20. Henderson W.R., Fukuyama K., Epstein W.L., and Spidler L.E.: Blocking of a cellular immune reaction to malignant melanoma by immunoglobulin from tumor-bearing animals. J. Reticul. Soc. 13:155-160, 1973.

21. Epstein W.L., Sagebiel R., Spidler L.E., Wybran J., Reed W., and Blois M.S.: Halo nevi and melanoma. JAMA 225:373-377, 1973.
22. Mozar H.n., Finnigan F.B., Petzoid H., Spidler L.E., Emmons R.W., and Rothenberg B.: Myelopathy following duck embryo rabies vaccine. JAMA 224:1605-1607, 1973.
23. Astor S.H., Spidler L.E., Frick O.L., and Fudenberg H.H.: Human leukocyte migration inhibition in agarose using four antigens: correlation with skin reactivity. J. Immunol. 110:1174-1179, 1973.
24. Levin A.S., Spidler L.E., and Fudenberg H.H.: Transfer factor therapy in immune deficiency states. Ann. Rev. Med. 24:175-208, 1973.
25. Spidler L.E., Levin A.S., and Fudenberg H.H.: Agammaglobulinemia absent delayed sensitivity and lymphopenia without infections: demonstration of immunologic unknowns. Am. J. Med. 54:371-377, 1973.
26. Bloom B.R., Ceppellini R., Cetotini J.C., David J.R., Kunkel H., Landy M., Lawrence H.S., Maini R., Nussenzweig V., Perlman P., Spidler L.E., Rosen F., and Zabriskie J.: In vitro methods of cell-mediated immunity: a progress report. Cell. Immunol. 6:331-347, 1973.
27. Levin A.S., Spidler L.E., and Fudenberg H.H.: Immune deficiency states. In Introduction to Clinical Allergy. Edited by B. Finegold, C.C. Thomas, Springfield, Illinois, 1973, pp. 346-359.
28. Hoffman P.M., Gaston D.D., and Spidler L.E.: Comparison of experimental allergic encephalomyelitis induced with spinal cord, basic protein, and synthetic encephalitogenic peptide. Clin. Immunol. and Immunopath. 1:364-371, 1973.
29. Wybran J., Levin A.S., Spidler L.E., and Fudenberg H.H.: Rosette-forming cells, immunologic deficiency diseases and transfer factor. New Engl. J. Med. 288:710-713, 1973.
30. Feigin R.D., Shackelford P.G., Eisen S., Spidler L.E., Pickering L.K., and Anderson D.C.: Treatment of mucocutaneous candidiasis with transfer factor. Pediatrics 53:63-70, 1974.
31. Spidler L.E., Levin A.S., and Fudenberg H.H.: Transfer factor. In Clinical Immunobiology, vol. 2. Edited by F.H. Bach and R.A. Good, Academic Press, New York, 2:153-175, 1974.
32. Fudenberg H.H., Levin A.S., Spidler L.E., Wybran J., and Byers V.: The therapeutic uses of transfer factor. Hosp. Prac. 9:95-104, 1974.

33. Catanzaro A., Spidler L.E., and Moser K.M.: Immunotherapy of coccidioidomycosis. J. Clin. Invest. 54:690-701, 1974.
34. Valdimarsson H., and Spidler L.E. (Chairman): Transfer factor, clinical application. In Progress in Immunology II, vol. 5. Edited by L. Brent and J. Holborow. North Holland Publication Company, Amsterdam, 1974, pp. 377-382.
35. Spidler L.E., Wybran J., Lieberman R., Levinson D., Epstein W., and Hendrickson C.: Results of intra-lesional BCG therapy in malignant melanoma: clinical and immunologic evaluation and complications. In Neoplasm Immunity: BCG Vaccination, Proceedings of a Chicago Symposium. Edited by R.G. Crispen, Institute for Tuberculosis Research, University of Illinois, Chicago, 1974, pp. 45-48.
36. Spidler L.E., Von Muller C. M., and Young J.D.: Experimental allergic encephalitis, study of cellular immunity to the encephalitogenic determinant. Cell. Immunol. 15:143-151, 1975.
37. Spidler L.E.: Transfer factor. Cutis Magazine 15:420-423, 1975.
38. Levin A.S., Byers V.S., Fudenberg H.H., Wybran J., Hackett A.J., Johnston J.O., and Spidler L.E.: Osteogenic sarcoma: immunologic parameters before and during immunotherapy with tumor-specific transfer factor. J. Clin. Invest. 55:487-499, 1975.
39. Catanzaro A., Spidler L.E., and Moser K.M.: Cellular immune response in coccidioidomycosis. Cell. Immunol. 15:360-371, 1975.
40. Spidler L.E., Spath P., Cooper N., and Fudenberg H.H.: Phagocytes and C4 in paraproteinemia. Brit. J. Haemat. 29:279-292, 1975.
41. Levin A.S., Spidler L.E., and Fudenberg H.H.: Transfer factor I: methods of therapy. In Immunodeficiency in Man and Animals: Birth Defects: Original Article, Series II. Edited by D. Bergsma. Sinauer Associates, Publishers, Sunderland, Massachusetts, 1975, pp. 445-448.
42. Spidler L.E., Levin A.S., and Fudenberg H.H.: Transfer factor II: results of therapy. In Immunodeficiency in Man and Animals: Birth Defects: Original Article, Series II. Edited by D. Bergsma. Sinauer Associates, Publishers, Sunderland, Massachusetts, 1975, pp. 449-456.

43. McKhan C.F., Hendrickson C.G., Spider L.E., Gunnarsson A., Banerjee D., and Nelson W.R.: Immunotherapy of melanoma with BCG: two fatalities following intra-lesional injection. Cancer 35:514-520, 1975.
44. Hoffman P.M., Spider L.E., Hsu M., and Fudenberg H.H.: Leukocyte migration inhibition in agarose. Cell. Immunol. 18:21-30, 1975.
45. Devich K.B., Lee J.C., Epstein W.L., Spidler L.E., and Hopper J., Jr.: Renal lesions accompanying poison oak dermatitis. Clin. Nephrol. 3:106-113, 1975.
46. Spidler L.E., Levin A.S., Stites D.P., Fudenberg H.H., and Huber H.: The Wiskott-Aldrich syndrome, immunologic studies in nine patients and selected family members. Cell. Immunol. 19:210-218, 1975.
47. Catanzaro A., and Spider L.E.: Transfer factor in diseases of the lung. In Immunologic and Infectious Reactions in the Lung. Edited by C.H. Kirkpatrick and H.Y. Reynolds. Marcel Dekker, New York, 1976, pp. 519-548.
48. Fidler I.J., and Spider L.E.: The effects of levamisole on in vivo and in vitro murine host response to syngeneic transplantation tumor. J. Natl. Cancer Inst. 55:1107-1112, 1975.
49. Olson J.A., Nelms D.C., Silverman S., and Spider L.E.: Levamisole, a new treatment for recurrent aphthous stomatitis. Oral Surg., Oral Med., Oral Path. 41:588-600, 1976.
50. Welch T.M., Triglia R., Spidler L.E., and Fudenberg H.H.: Preliminary studies on human "transfer factor" activity in guinea pigs: systemic transfer of cutaneous delayed-type hypersensitivity to PPD and SKSD. Clin. Immunol. Immunopathol. 5:407-415, 1976.
51. Hoffman P.M., Spider L.E., and Hsu M.: Leukocyte migration inhibition in guinea pigs. 1. Correlation with skin test reactivity and macrophage-migration inhibition. Cell. Immunol. 21:358-363, 1976.
52. Spider L.E., Levin A.S., and Wybran J.: Combined immunotherapy in malignant melanoma: regression of metastatic lesions in two patients concordant in timing with systemic administration of transfer factor and bacillus Calmette-Guerin. Cell. Immunol. 21:1-19, 1976.
53. Levinson A.I., Hopewell P.C., Stites D.P., Spidler L.E., and Fudenberg H.H.: Co-existent lymphoid interstitial pneumonia, pernicious anemia, and agammaglobulinemia. Comment on autoimmune pathogenesis. Arch. Intern. Med. 136:312-316, 1976.

54. Spidler L.E.: Delayed hypersensitivity skin testing. In Manual of Clinical Immunology. Edited by N.R. Rose and H. Friedman. American Society for Microbiology, Washington, D.C., 1976, pp. 53-63.
55. Catanzaro A. and Spidler L.E.: For the Coccidioidomycosis Cooperative Treatment Group: Clinical and immunologic results of transfer factor therapy in coccidioidomycosis. In Transfer Factor Basic Properties and Clinical Applications. Edited by M.S. Ascher, A.A. Godieb and C.H. Kirkpatrick, Academic Press, New York, 1976. pp 477-491.
56. Spidler L.E.: Malignant melanoma. J. Invest. Dermatol. 67:435-441, 1976.
57. Dau P.C., Johnson K.P., and Spidler L.E.: The effect of levamisole on cellular immunity in multiple sclerosis. Clin. Exp. Immunol. 26:302-309, 1976.
58. Wybran J., Spidler L.E., Lieberman R., and Fudenberg H.H.: "Active" T-cell rosettes and total T-rosettes in patients with melanoma following intratumoral inoculation of BCG: a clue to the mechanism of action of baccillus Calmette-Guerin? Cancer Immunol. Immunother. 1:153-156, 1976.
59. Triglia R., and Spidler L.E.: Human "transfer factor" activity in guinea pigs. Further studies. In Transfer Factor. Basic Properties and Clinical Applications. Edited by M.S. Ascher, A.A. Gottlieb and C.H. Kirkpatrick, Academic Press, New York, 1976, pp. 695-703.
60. Spidler L.E., and Von Muller C.: Leukocyte migration inhibition in agarose. In In Vitro Methods in Cell-Mediated and Tumor Immunity. Edited by B. Bloom and J.R. David. Academic Press, New York, 1976, pp. 645-650.
61. Stevens D.A., et al.: (Coccidioidomycosis Cooperative Treatment Group): Transfer factor therapy of infectious diseases. In Advances in Dermatopharmacology (Chapter 6). Edited by P. Frost, E.C. Gomez and N. Zaias, Spectrum Publications, 1977, pp. 67-74.
62. Spidler L.E., Glogau R., Nelms D., Silverman S., Jr., Olson J., O'Conner R., Osder H., Smolin G., Basch K., Wong P., Engleman E.P., and Brugmans J.: Clinical and immunological effects of levamisole. In Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasias. (Fogarty International Center Proceedings 28). Edited by M.A. Chirigos, U.S. Government Printing Office, Washington, D.C., 1974, pp. 71-75.
63. Ibrahim A.B, Triglia R., Dau P.C., and Spidler L.E.: Anti-tumor effects of levamisole on an allogeneic hamster melanoma and a syngeneic rat hepatoma. In Progress in Cancer

Research, vol. 2. Control of Neoplasia by Modulation of the Immune System. Edited by M.A. Chirigos, Raven Press, New York, 1977.

64. Spider L.E., and Dau P.C.: Immunotherapy in infectious disease, autoimmunity and cancer. Acta Neurol. Scand. (Suppl. 63):227-233, 1977.
65. Spider L.E., Glogau R.G., Nelms D.C., Basch C.M., Olson J.A., Silverman S., Jr., and Engleman E.P.: Clinical and immunologic effects of levamisole. In Progress in Cancer Research, vol. 2. Control of Neoplasia by Modulation of the Immune System. Edited by M.A. Chirigos, Raven Press, New York, 1977, pp. 217-225.
66. Spider L.E., Littooy F.N., and Sagebiel R.W.: Cellular immunity in patients with malignant melanoma and their household contacts. Cancer Immunol. Immunother. 2:69-76, 1977.
67. Silverman S., Jr., Olson J.A., Nelms D.C., and Spider L.E.: Recurrent aphthous stomatitis: current status of etiology and treatment. J. Calif. Dental Assoc. 2/1977, pp. 38-44.
68. Wolf R.E., Fudenberg H.H., Welch T.M., Spider L.E., and Ziff M.: Treatment of Becher's syndrome with transfer factor. JAMA 238:869-871, 1977.
69. Spivack S.D., Spider L.E., and Dunphy J.E.: Oncology and cancer chemotherapy. In Current Surgical Diagnosis and Treatment. Edited by J.E. Dunphy and L.W. Way, Lange Medical Publications, Los Altos, California, 1977, pp. 1059-1082.
70. Dau P.C., Lindstrom J.M., Cassel C.K., Denys E.H., Shev E.E., and Spider L.E.: Plasmapheresis and immunosuppressive drug therapy in myasthenia gravis. New Engl. J. Med. 297:1134-1140, 1977.
71. Berkel A.I., Ersoy F., Epstein L.B., and Spider L.E.: Transfer factor therapy in ataxia-telangiectasia. Clin. Exp. Immunol. 29:376-384, 1977.
72. Basch C.M., Spider L.E., Engleman E.G., and Engleman E.P.: Cellular immune reactivity in patients with rheumatoid arthritis and effects of levamisole. J. Rheumatol. 4:377-388, 1977.
73. Spider L.E.: Transfer factor. Int. J. Dermatol. 17:445-458, 1978.
74. Spider L.E., Sagebiel R.W., Wong P.P., Malm T.M., Chase R.H., and Gonzalez R.L.: A randomized double-blind trial of adjuvant therapy with levamisole versus placebo in patients with malignant melanoma. In Progress in Cancer Research and Therapy, vol. 6.

Immunotherapy of Cancer: Present Status of Trials in Man. Edited by W.D. Terry and D. Windhorst, Raven Press, New York, 1978, pp. 73-79.

75. Spider L.E., Wong P., and Sagebiel R.: Combined immunotherapy of malignant melanoma-unusual survival following cerebral metastasis. Arch. Dermatol. 114:1501, 1978.
76. Gross P.A., Patel C., and Spider L.E.: Disseminated cryptococcus treated with transfer factor. JAMA 240:2460-2462, 1978.
77. Suquet Von Muller C., Spider L.E., and LeCocq J.: Experimental allergic encephalitis: study of cellular immunity during disease suppression. Eur. J. Immunol. 8:771-776, 1978.
78. Spider L.E.: Transfer factor. In Handbook Series in Clinical Laboratory Science. Section F: Immunology, vol. 1, part 1. Edited by A. Baumgarten and F.F. Richards, CRC Press, West Palm Beach, Florida, 1978, pp. 87-106.
79. Gonzalez R.L., Spider L.E., and Sagebiel R.W.: Effect of levamisole as a surgical adjuvant therapy for malignant melanoma. Cancer Treatment Reports 62:1703-1707, 1978.
80. Spider L.E., Mischak R., and Basch C.: Pharmacological modification of suppressor cell activity. In The Pharmacology of Immuregulation. Edited by G.H. Werner and F. Floch, Academic Press, New York, 1978, pp. 239-251.
81. Mischak R.P., Dau P.C., Gonzalez R.L., and Spider L.E.: In vitro testing of suppressor cell activity in myasthenia gravis. In International Conference on Plasmapheresis and the Immunobiology of Myasthenia Gravis. Edited by P.C. Dau; Clark, Houghton, Mifflin, New York, 1979, pp. 72-78.
82. Gonzalez R.L., Wong P., and Spider L.E.: Altered regulation of mitogen responsiveness by suppressor cells in multiple sclerosis. Clin. Exp. Immunol. 36:78-84, 1979.
83. Jonas S., Wichter M., and Spider L.E.: Amyotrophic lateral sclerosis: failure of transfer factor therapy. Ann. Neurol. 67:59-66, 1979.
84. Spider L.E.: Transfer factor therapy in the Wiskott-Aldrich syndrome: results of long-term follow-up in 32 patients. Am. J. Med. 67:59-66, 1979.
85. Spivack S.D., Spider L.E., Holm D.C., and Dunphy J.E.: Oncology and cancer chemotherapy. In Current Surgical Diagnosis and Treatment (Chapter 49). Edited by S.D.

- Spivack and L.W. Way, Lange Medical Publications, Los Altos, California, 1979, pp. 1073-1101.
86. Spitler L.E.: Transfer factor in immunodeficiency diseases. Ann. N.Y. Acad. Sci. 332:228-235, 1979.
  87. Gonzalez R.L., Wong P., and Spitler L.E.: Adjuvant immunotherapy with transfer factor with melanoma metastatic to lung. Cancer 45:57-63, 1980.
  88. Spitler L.E.: Transfer factor: Failure to transfer reactivity in normal human subjects. Clin. Exp. Immunol. 39:708-716, 1980.
  89. Spitler L.E.: BCG, levamisole, and transfer factor in the treatment of cancer. In Current Cancer Immunology, Progress in Experimental Tumor Research, vol. 25. Edited by V. Richards, Karger Publications, New York, 1980, pp. 178-193.
  90. Lozada F., Spitler L.E., and Silverman S., Jr.: Results of immunologic testing in patients with erythema multiforme. J. Dent. Res. 59:567-572, 1980.
  91. Lozada R., Spitler L.E., and Silverman S., Jr.: Clinical immunologic responses to levamisole in 13 patients with erythema multiforme. Int. J. Immunopharmacol. 2:63-68, 1980.
  92. Spitler L.E., Wray B.B., Mogerman S., Miller, III, J.J., O'Reilly R.J., and Lagios M.: Nephropathy in the Wiskott-Aldrich syndrome. Pediatrics, vol. 66, pp. 391-398, 1980.
  93. Spitler L.E., and Sagebiel R.W.: A randomized trial of levamisole versus placebo as adjuvant therapy in malignant melanoma. New Engl. J. Med. 303:1143-1147, 1980.
  94. Spitler L.E.: Delayed hypersensitivity skin testing. In Manual of Clinical Immunology, 2nd edition. Edited by N.R. Rose and H. Friedman. American Society of Microbiology, Washington, D.C., 1980, pp. 200-212.
  95. Owen R.L., Dau P.C., Johnson K.P., and Spitler L.E.: Immunologic mechanisms in multiple sclerosis: exacerbation by type A hepatitis and by skin test antigens. JAMA 244:2307-2309, 1980.
  96. Fisher G.L., Spitler L.E., McNeil K.L., and Rosenblatt L.S.: Serum copper and zinc levels in melanoma patients. Cancer 47(7):1837-1844, 1981.



97. de Merieùx P., Spidler L.E., and Paulus H.E.: Treatment of Bechets syndrome with levamisole. Arth. Rheu. 24(1):64-70, 1981.
98. Catanzaro A., Spidler L.E., Campbell G.D., and Moser K.M.: Transfer factor for histoplasmosis in a patient with Hodgkin's disease. Arch. Intern. Med. 141:533-537, 1981.
99. Spidler L.E., Sagebiel R.W., Allen R., Minor D., Dymott C., and Drake T.: Levamisole in the treatment of melanoma. Immunother. Human Cancer, section VI, pp. 289-291, 1982.
100. Spidler L.E., and Scott C.F.: Immunotherapy of melanoma. In Melanoma Antigens and Antibodies. Edited by R.A. Reisfeld and S. Ferrone, Plenum Press, New York and London, 1982, pp. 355-363.
101. Spidler L.E., and Scott C.F.: Lymphocytotoxic antibody in multiple sclerosis: activity against T-cell subsets and correlation with disease activity. In Clin. Exp. Immunol. 53:133-139, 1983.
102. Scott C.F., Cashman N., and Spidler L.E.: Experimental allergic encephalitis: treatment with drugs which alter CNS serotonin levels. J. Immunopharmacol. 4(3):153-162, 1982-83.
103. Levy J.A., Lee H.M., Kawahata R.T., and Spidler L.E.: Purification of monoclonal antibodies from mouse ascites eliminates contaminating infectious mouse type C virus and nucleic acids. In Clin. Exp. Immunol. 56:114-120, 1984.
104. Englestad B., Khentigan A., and Spidler L.E.: Clinical radioimmunoimaging. In Diagnostic Radiology. Edited by A. Margulis and C. Gooding, RREF Press, San Francisco, 1985, pp. 525-532.
105. Spidler L.E.: Removal of xenotropic and ecotropic viruses by purification of monoclonal antibodies. In Abnormal Cells, New Products and Risk. (In vitro cellular and developmental biology. Monograph; n.6). Proceedings of a workshop held July 30-31, 1984 at the National Institutes of Health, Bethesda, Maryland. Edited by H.E. Hopps and J.C. Petricciani, Tissue Culture Association, Gaithersburg, MD, 1985, pp.121-134.
106. Englestad B.L., Spidler L.E., del Rio M., Ramos E.C., Rosendorf L.L., Reinhold C.E., Khentigan A., Huberty J.P., Corpuz S.W., Lee H.M., Okerlund M.D., Hamner R.S., and Scannon P.J.: Phase I immunolymphoscintigraphy with an indium 111-labeled-antimelanoma monoclonal antibody. Radiology 161:419-422, 1986.

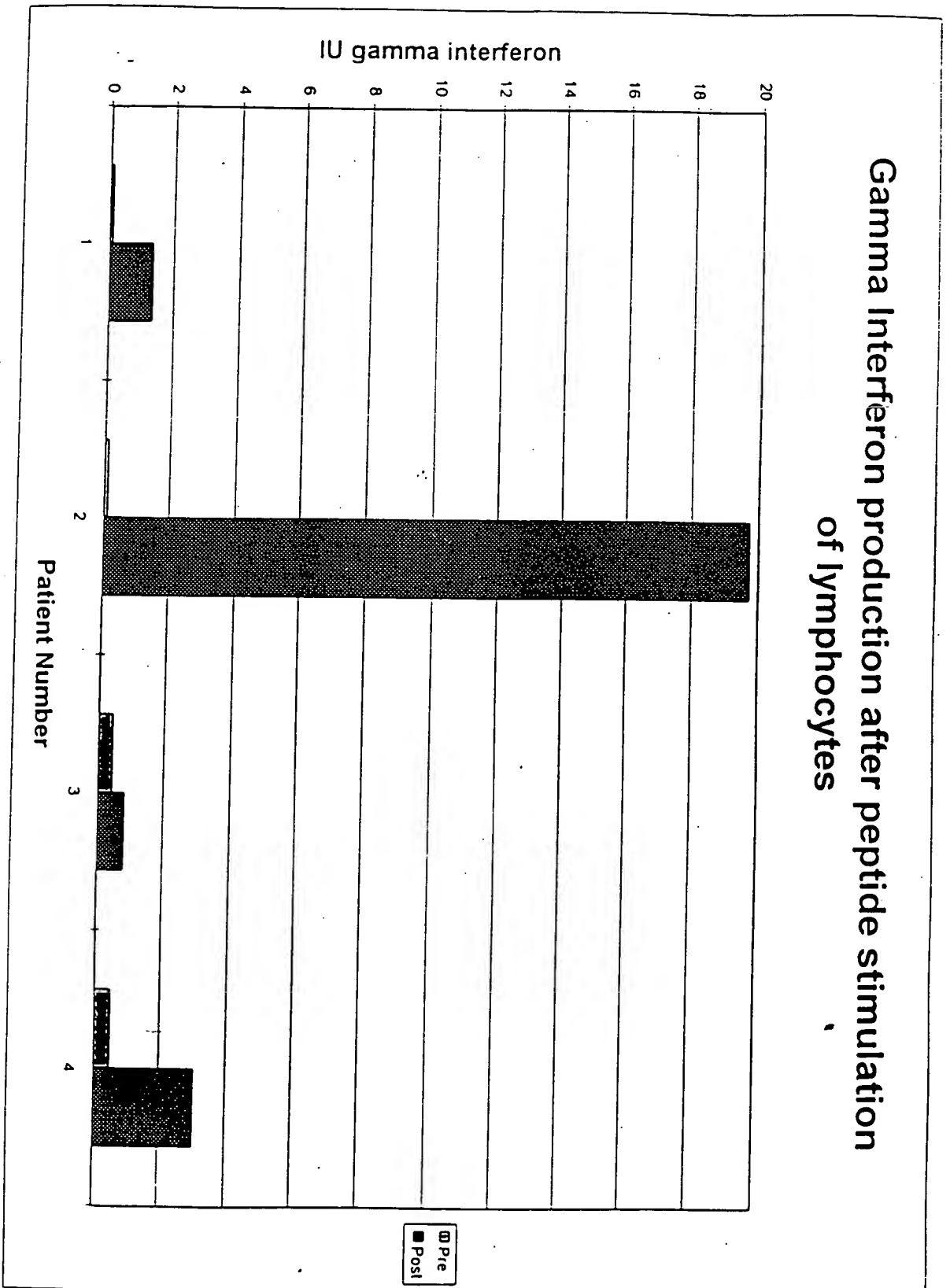
107. Engelstad B.C., Ramos E.C. Stoudemire J., O'Connell J.W., Villanueva J., Faulkner D.B., Hamner R.S., Spidler L.E., and Scannon P.: Improved immunespecificity in monoclonal radioummunoimaging using dual radionuclide color functional maps. Investigative Radiology 21:917-921, 1986.
108. Spidler L.E.: Immunotoxin therapy of malignant melanoma. Med. Oncol. & Tumor Pharmacother. 3:147-152, 1986.
109. Spidler L.E., del Rio M., Khentigan A., Wedel N.I., Brophy N.A., Miller L., Harkonen W.S., Rosendorf L., Lee H.M., Mischak R.P., Kawahata R.T., Stoudemire J.B., Fradkin L.B., Bautista E.E., and Scannon P.J.: Therapy of patients with malignant melanoma using a monoclonal antimelanoma antibody-ricin A chain immunotoxin. Cancer Research 47:1717-1723, 1987.
110. Harkonen S., Stoudemire J., Mischak R.P., Spidler L.E., Lopez H., and Scannon P.: Toxicity and immunogenicity of monoclonal antimelanoma antibody ricin A chain immunotoxin in rats. Cancer Research 47:1377-1382, 1987.
111. Spidler L.E.: Phase I clinical trials with immunotoxins. Immunoconjugates: Antibody Conjugates in Radioimaging and Therapy of Cancer. Edited by C.W. Vogel, Oxford University Press, New York, 1987, pp.290-300.
112. Herder, R.A., Spidler, L.E., and Frankel, A.E.: Humoral immune response to ricin A chain immunotoxin in patients with metastatic melanoma. Cancer Drug Delivery 4:245 - 253, 1987
113. Miller L.L, Spidler L.E., Allen R.A., and Minor D.R.: A randomized, double-blind, placebo controlled trial of transfer factor as adjuvant therapy for malignant melanoma. Cancer, 61:1543-1549, 1988.
114. Spidler, L.E.: Clinical Studies: Solid Tumors. Immunotoxins. Edited by Arthur E. Frankel, Martinus Nijhoff B.V., Boston, 1988 pp. 491-512.
115. Spidler L.E., and Miller L.: Clinical trials of transfer factor in malignancy. J. Exp. Pathol. 3:549-564, 1988
116. Harkonen S., Scannon P., Mischak R.P., Spidler L.E., Foxall C., Kennedy D., and Greenberg R.: Phase I study of a murine monoclonal anti-lipid A antibody in bacteremic and non-bacteremic patients. Antimicrobial Agents and Chemotherapy, 710-716, 1988.

117. LoBuglio A.F., Khazaeli M.B., Lee J., Haynes A., Sumerel L., Mischak R.P., and Spidler L.E. Pharmacokinetics and immune response to Xomazyme-Mel in melanoma patients. Antibody Immunoconjugates and Radiopharmaceuticals 1:305 - 310, 1988.
118. Spidler, L.E., Mischak, R. and Scannon, P.: Therapy of metastatic malignant melanoma using Xomazyme-Mel, a murine monoclonal antimelanoma ricin A chain immunotoxin. Nuclear Medicine and Biology International Journal of Applied Radiation and Isotopes, Part B, 16(6):625-627, 1989.
119. Spidler, L.E.: Monoclonal Antibodies In the Treatment of Malignant Melanoma. The Present and Future Role of Monoclonal Antibodies in the Management of Cancer. Frontiers of Radiation Therapy and Oncology, 24, 186-193, 1989
120. Mischak, R.P., Foxall, C., Rosendorf, L.L., Knebel, K., Scannon, P.J., Spidler, L.E.: Human antibody responses to components of the monoclonal antimelanoma antibody ricin A chain immunotoxin Xomazyme [registered trademark] - MEL. Molecular Biotherapy, 2:104-109, 1990.
121. Stoudemire, J.B., Mischak, R., Foxall, C., Harkonen, W.S., Del Rio, M., and Spidler, L.E.: The effects of cyclophosphamide on the toxicity and immunogenicity of ricin A chain immunotoxin in rats. Molecular Biotherapy, 2: 179-184, 1990.
122. Oratz, R., Speyer, J.L., Wernz, J.C., Hochster, H., Meyers, M., Mischak, R., and Spidler, L.E.: Antimelanoma monoclonal antibody - ricin A chain immunoconjugate (XMMME-0001-RTA) plus cyclophosphamide in the treatment of metastatic malignant melanoma: Results of a phase II trial. J. Biol Response Mod., 9(4): 345-354, 1990.
123. Spidler, L.E.: Immunotoxins. In: Principles of Cancer Biotherapy. Edited by R.K. Oldham, Marcel Dekker, inc., New York, pp 433-456, 1991.
124. Spidler, L.E.: A randomized trial of levamisole versus placebo as adjuvant therapy in malignant melanoma. Journal of Clinical Oncology, 9: 736-740, 1991.
125. Gonzalez, R., Salem, P., Bunn Jr., P.A., Zukowski, A.A., Lamb, R., Benjamin, R.S., Spidler, L., Wedel, N., and Robinson, W.A. Single-dose murine monoclonal antibody ricin A chain immunotoxin in the treatment of metastatic melanoma: a phase I trial. Mol. Biother., 3:192-196, 1991.
126. Spidler, L.E., Conjugated Antibodies: Immunotoxins. In Antibody Therapy of Cancer. Edited by Robert Dillman, Marcel Dekker, New York, In Press, 1992.

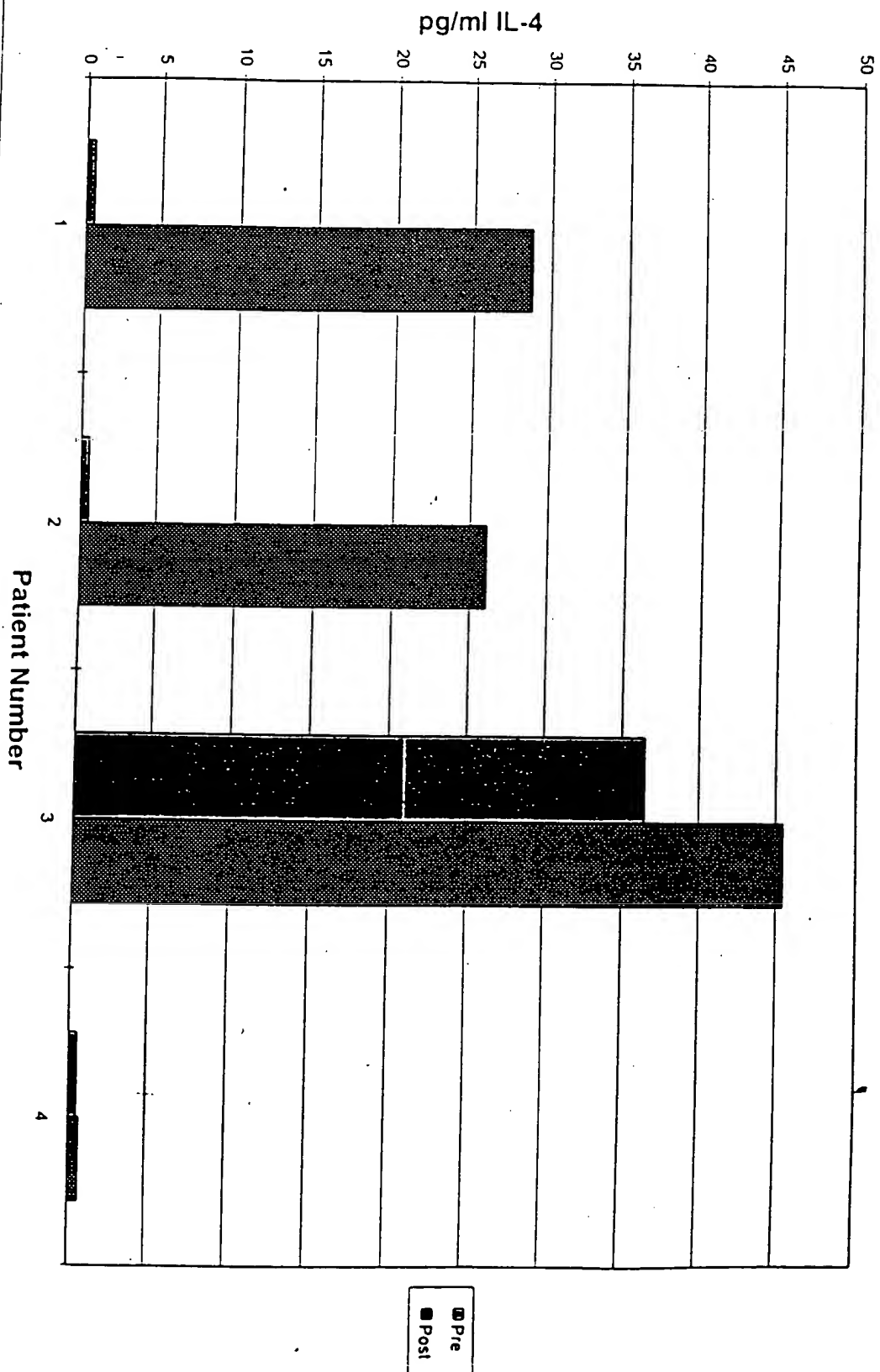
127. Spidler, L.E. and Munoz, A.: Value of percentage of lymphocytes and active rosettes in determination of prognosis in malignant melanoma. In preparation, 1992.
128. Spidler, L.E., Von Wussow, P., Carey, R.W., Borden, E., Benjamin, R.S., Oratz, R., Tseng Jr., A., Mastrangelo, M., and Ernstoff, M.S.: Phase II Trial of a monoclonal antimelanoma antibody Ricin A chain immunotoxin in therapy of malignant melanoma. Journal of Clinical Oncology, in preparation.
129. Spidler, L.E., and Minor, D.R.: Monoclonal antimelanoma ricin A chain immunotoxin therapy of metastatic melanoma in an outpatient setting. Journal of Clinical Oncology, in preparation.

*Revised January, 1992*

## Gamma Interferon production after peptide stimulation of lymphocytes

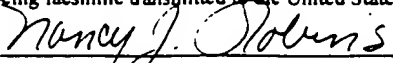


# IL-4 production after peptide stimulation of lymphocytes



CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on November 4, 1996.

  
Nancy J. Robins

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Lynn E. Spitler et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

DECLARATION OF PHILIP O. LIVINGSTON, MD  
PURSUANT TO 37 C.F.R § 1.132

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, Philip O. Livingston, MD, declare as follows:

1. I am a Associate Professor and in charge of the laboratory of Developmental Tumor Vaccinology at Memorial Sloan-Ketter Cancer Center in New York, New York. I am a member of the Scientific Advisory Board for Jenner Technologies, the assignee of this application, and am also a shareholder in the company. A copy of my *Curriculum Vitae* is attached hereto as Exhibit A.

2. I have reviewed the Declaration Under 37 C.F.R. 1.132 prepared by Dr. Lynn E. Spitler describing the results of a clinical study directed to the use of prostate specific antigen

(PSA) as an active ingredient in an antiprostate cancer vaccine. I am also familiar with the study itself, and with the results that were obtained.

3. The purpose of the study was to obtain initial evidence the vaccines would raise a sufficient cellular immune response to have a beneficial effect with respect to prostate tumors. Such a result could be shown directly by measuring cytotoxic lymphocyte (CTL) generation; however, I am aware that this was not possible in these studies because of problems assaying cytotoxic T cell activity. This problem is widespread in the field, despite occasional reports to the contrary, and a lack of sensitive assays for CTL activity is widely considered to be one of the major obstacles to the development of a new generation of vaccines capable of inducing cytotoxic T cells against tumors. This is due to uncertainty over the optimal assay, the optimal time from immunization to blood drawing, and whether testing for CTL activity in the peripheral blood lymphocytes would ever be capable of reflecting the systemic induction of effective CTLs. Consequently, other assays for T cell immunity have been widely used..

4. The responses measured are understood in the art to be satisfactory substitutes for measuring CTLs. Thus, the proliferation of lymphocytes from two of the patients in response to contact with PSA or in response to peptides representing putative PSA epitopes suggests an appropriate cellular immune response. The ability of PSA or PSA derived peptides to stimulate cytokine production -- i.e., gamma interferon and IL-4 production -- from lymphocytes in these patients indicates that the cellular response is obtained specifically with respect to PSA. The observation of the development of a positive skin test response to PSA in one patient is also consistent with these observations showing the development of cell mediated immunity in this patient.

5. In my opinion, the results obtained in this clinical study provide evidence that the vaccines are likely to be effective in exerting a beneficial effect on patients with prostate tumors or at risk for prostate tumors, though much further work will be required to increase the frequency and potency of the responses..

6. The efficacy shown for the vaccine tested in the foregoing clinical studies further provides evidence that analogous vaccines based on host tissue antigen, such as prostate specific membrane antigen (PSMA) and prostate acid phosphatase (PAP) would behave in a similar



manner. It is also well known that if the entire antigen is effective as a vaccine, portions of the antigen will be effective as well, especially if manipulated by art-known methods to enhance their immunogenicity, such as by coupling them to carrier.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

October 14, 1996

Philip O. Livingston, MD

CURRICULUM VITAE

NAME: Philip O. Livingston, M.D.

DATE OF BIRTH: January 14, 1943

PLACE OF BIRTH: New York, N.Y.

NATIONALITY: U.S.A.

EDUCATION: Princeton University, Princeton, New Jersey  
B.A. Biology - 1965  
  
Harvard Medical School, Boston, Massachusetts  
M.D. - 1969

HOSPITAL APPOINTMENTS: Cornell Cooperating Hospitals, New York  
Intern 7/1/69 - 6/30/70  
  
Cornell Cooperating Hospitals, New York  
Resident 7/1/70 - 6/30/71  
  
New York University Hospital, New York  
Fellow - Immunology, 7/1/71 - 6/30/73  
  
U.S. Naval Hospital at Roosevelt Road, Puerto Rico  
Lt. Cmdr., Chief of Allergy and Rheumatology Services  
1973 - 1975  
  
Memorial Hospital, New York - Fellow -  
Department of Medicine - Clinical Immunology Service  
7/1/75 - 6/30/77  
  
Memorial Hospital, New York - Assistant Attending  
Physician - Department of Medicine  
Clinical Immunology Service  
7/1/77 - 7/25/90  
  
Memorial Hospital, New York - Associate Attending  
Physician - Department of Medicine  
Clinical Immunology Service  
7/26/90 - Present

RESEARCH APPOINTMENTS: Memorial Sloan-Kettering Cancer Center, New York  
  
Research Fellow 7/1/75 - 6/30/77  
Research Associate 7/1/77 - 1/1/85  
Assistant Member 1/2/85 - 7/25/90  
Associate Member 7/26/90 - Present

TEACHING APPOINTMENTS: Cornell University Medical College  
Assistant Professor of Medicine  
7/1/77 - 1985  
  
Associate Professor 7/1/93 - Present

*Exhibit A*

HONORS AND FELLOWSHIPS: American Cancer Society Junior Faculty  
Clinical Fellow, July 1978 - 1981

BOARD CERTIFICATIONS: American Board of Internal Medicine 1972,  
recertified 1981:  
Subspecialty board in Allergy and  
Immunology, 1973 and Medical Oncology, 1982.

HOME ADDRESS: 156 East 79th Street  
New York, N.Y. 10021  
(212) 472-3390

OFFICE ADDRESS: 1275 York Avenue  
New York, N.Y. 10021  
(212) 639-7425

SOCIAL SECURITY: 046-34-7719

MARITAL STATUS: Married SPOUSE'S NAME: Lucy Hann, M.D.

DEPENDENTS: 1

MILITARY STATUS: Lt. Cmdr. U.S. Navy 1973-1975

PUBLICATIONS:

Livingston, P.O., Shiku, M., Bean, M.A., Pinsky, C.M., Oettgen, H.F. and Old, L.J.: Cell-mediated cytotoxicity for cultured autologous melanoma cells. Int. J. Cancer 24: 34-44, 1979.

Livingston, P.O., Michitsch, R., Shiku, H., Pinsky, C.M. and Oettgen, H.F.: Antibody-dependent cell-mediated cytotoxicity for cultured autologous melanoma cells. Cell Imm. 64: 131-143, 1981.

Pollack, M.S., Livingston, P.O., Fogh, J., Carey, T.E., Oettgen, H.F. and Dupont, B.: Genetically appropriate expressions of HLA and DR (IA) alloantigens on human melanoma cell lines. Tissue Antigens 15: 249-254, 1980.

Pollack, M.S., Heagney, S.D., Livingston, P.O. and Fogh, J.: HLA-A, B, C and DR allogeneic expression on 46 cultured human tumor cell lines. J. Clin. Immunol. 66: 1003-1012, 1981.

Watanabe, T. and Livingston, P.O.: Establishment and maintenance of cultured tumor cell lines and maintenance of cultured amniotic cells for use in HLA typing. IN: AACHT Tissue Typing Manual, Zachary, A.A. and Braun, W.E. (eds.), AACHT, New York, I-21-1 - I-21-4, 1981.

Livingston, P.O., Watanabe, T., Shiku, H., Houghton, A.N., Albino, A., Takahashi, T., Resnick, A., Michitsch, R., Pinsky, C.M., Oettgen, H.F. and Old, L.J.: Serological response of melanoma patients receiving melanoma cell vaccines, I. Autologous cultured melanoma cells. Int. J. Cancer 30: 413-422, 1982.

Livingston, P.O., Oettgen, H.F. and Old, L.J.: Specific active immunotherapy in cancer therapy. IN: Immunological Aspects of Cancer Therapeutics, Dr. Enrico Mihich (ed.), John Wiley and Sons, Inc. Publ., 363-404, 1982.

Livingston, P.O., Takeyama, H., Pollack, M.S., Houghton, A.N., Albino, A., Pinsky, C.M., Oettgen, H.F. and Old, L.J.: Serological responses of melanoma patients to vaccines derived from allogeneic cultured melanoma cells. Int. J. Cancer 31: 567-575, 1983.

Livingston, P.O., DeLeo, A.B., Jones, M., Oettgen, H.F. and Old, L.J.: Comparison of approaches for augmenting the serological response to the Meth A antigen. Pretreatment with Cyclophosphamide is most effective. J. Immunol. 131: 2601, 1983.

Livingston, P.O., Albino, A.P., Chung, T.J.C., Real, F.X., Houghton, A.N., Oettgen, H.F. and Old, L.J.: Serological response of melanoma patients to vaccines prepared from VSV lysates of autologous and allogeneic cultured melanoma cells. Cancer 55: 713-720, 1985.

Livingston, P.O., Kaelin, E., Pinsky, C.M., Oettgen, H.F. and Old, L.J.: IV. Serological response in stage II melanoma patients receiving allogeneic melanoma cell vaccines. Cancer 56: 2194-2200, 1985.

Livingston, P.O., Jones, M., DeLeo, A.B., Oettgen, H.F. and Old, L.J.: The serological response to Meth A sarcoma vaccines after Cyclophosphamide treatment is further increased by various adjuvants. J. Immunol. 135:1505-1509, 1985.

Livingston, P.O., Old, L.J. and Oettgen, H.F.: Approaches to augmenting the immunogenicity of tumor antigens. IN: Monoclonal Antibodies and Cancer Therapy, UCLA Symposia on Molecular and Cellular Biology, New Series, Volume 27, R.A. Reisfeld and S. Sell (eds.), Alan R. Liss, Inc., New York, N.Y., 537-550, 1985.

Oettgen, H.F., Livingston, P.O., Takahashi, T., Shiku, H., Watanabe, T., Takeyama, H., Houghton, A.N., Albino, A., Resnick, L., Lloyd, K. and Old, L.J.: Specific active immunization against cell surface antigens of human malignant melanoma. Development of an approach. IN: Advances in Immunopharmacology, Hadden, J. (ed.). Pergamon Press, Oxford, 235-242, 1981.

Pollack, M.S. and Livingston, P.O.: HLA and DR antigen frequencies in melanoma patients: Possible relation to disease prognosis. Tissue Antigens 26: 262-265, 1985.

Natoli, Jr., E.J., Livingston, P.O., Cordon-Cardo, C., Pukel, C.S., Lloyd, K.O., Wiegandt, H., Szalay, J., Oettgen, H.F. and Old, L.J.: A murine monoclonal antibody detecting n-acetyl and n-glycolyl GM2: Characterization of cell surface reactivity. Cancer Res. 46: 4116-4120, 1986.

Livingston, P.O., Jones Calves, M. and Natoli, Jr., E.J.: Approaches to augmenting the immunogenicity of the ganglioside GM2 in mice: Purified GM2 is superior to whole cells. J. Immunol. 138: 1524-1529, 1987.

Livingston, P.O., Natoli, Jr., E.J., Jones Calves, M., Stockert, E., Oettgen, H.F. and Old, L.J.: Vaccines containing purified GM2 ganglioside elicit GM2 antibodies in melanoma patients. Proc. Natl. Acad. Sci. USA 84: 2911-2915, 1987.

Livingston, P.O., Cunningham-Rundles, S., Marfleet, G., Gnecco, C., Wong, G.Y., Schiffman, G., Enker, W.E. and Hoffmann, M.K.: Inhibition of suppressor cell activity by cyclophosphamide in patients with malignant melanoma. J. Biol. Resp. Mod. 6: 392-403, 1987.

Yamaguchi, H., Furukawa, K., Fortunato, S., Livingston, P.O., Lloyd, K.O., Oettgen, H.F. and Old, L.J.: Cell-surface antigens of melanoma recognized by human monoclonal antibodies. Proc. Natl. Acad. Sci. USA 84: 2416-2420, 1987.

Livingston, P.O.: Experimental and clinical studies with active specific immunotherapy. Immunity to Cancer II, M.S. Mitchell (ed.), Alan R. Liss Inc., New York, N.Y. pp. 309-321, 1989.

Livingston, P.O.: The basis for ganglioside vaccines in melanoma. IN: Human Tumor Antigens and Specific Tumor Therapy, UCLA Symposia on Molecular and Cellular Biology, Volume 99, R. Metzgar and M. Mitchell (eds.), Alan R. Liss. Inc., New York, N.Y. pp. 287-296, 1989.

Livingston, P.O., Ritter, G., Oettgen, H.F. and Old, L.J.: Immunization of melanoma patients with purified gangliosides. IN: Gangliosides in Cancer, H.F. Oettgen (ed.), VCH Verlagsgesellschaft, Weinheim, Germany, pp. 293-299, 1989.

Ritter, G., Livingston, P.O., Boosfeld, E., Wiegandt, H., Yu, R.K., Oettgen, H.F. and Old, L.J.: Development of melanoma vaccines: gangliosides as immunogens. IN: Gangliosides in Cancer, H.F. Oettgen (ed.), VCH Verlagsgesellschaft, Weinheim, Germany, pp. 301-313, 1989.

Livingston, P.O., Ritter, G. and Calves, M.J.: Antibody response after immunization with the gangliosides GM1, GM2, GM3, GD2 and GD3 in the mouse. Cancer Immunol. Immunother. 29: 179-184, 1989.

Livingston, P.O., Ritter, G., Srivastava, P., Padavan, M., Calves, M.J., Oettgen, H.F. and Old, L.J.: Characterization of IgG and IgM antibodies induced in melanoma patients by immunization with purified GM2 ganglioside. Cancer Res. 49: 7045-7050, 1989.

Ritter, G., Boosfeld, E., Markstein, E., Yu, R.K., Ren, S., Oettgen, H.F., Old, L.J. and Livingston, P.O.: Biochemical and serological characteristics of natural 9-O-acetyl GD3 from human melanoma and bovine buttermilk and chemically O-acetylated GD3. Cancer Res. 50: 1403-1410, 1990.

Yamaguchi, H., Furukawa, K., Fortunato, S.R., Livingston, P.O., Lloyd, K.O., Oettgen, H.F. and Old, L.J.: A human monoclonal antibody with dual GM2/GD2 specificity derived from an immunized patient. Proc. Natl. Acad. Sci. USA 87: 3333-3337, 1990.

Ritter, G., Boosfeld, E., Calves, M.J., Oettgen, H.F., Old, L.J. and Livingston, P.O.: Antibody response after immunization with gangliosides GD3, GD3 lactones, GD3 amide and GD3 gangliosidol in the mouse. GD3 lactone I induces antibodies reactive with human melanoma. Immunobiology 182: 32-43, 1990.

Livingston, P.O.: Active specific immunotherapy in the treatment of cancer. IN: Immunology and Allergy Clinics of North America: Human Cancer Immunology II, H.F. Oettgen (ed.), W.B. Saunders Company, London, U.K., Volume 11:2, pp. 402-423, 1991.

Ritter, G., Boosfeld, E., Adluri, R., Calves, M., Oettgen, H.F., Old, L.J. and Livingston, P.O.: Antibody response to immunization with ganglioside GD3 and GD3 congeners (lactones, amide and gangliosidol) in patients with malignant melanoma. Int. J. Cancer 48: 379-385, 1991.

Ritter, G. and Livingston, P.O.: Cancer associated ganglioside antigens. IN: Seminars in Cancer Biology, M. Longenecker (ed.), W.B. Saunders, London, U.K., Vol. 2: 401-409, 1991.

Oettgen, H.F., Livingston, P.O. and Old, L.J.: Melanoma. IN: Biologic Therapy of Cancer, V.T. DeVita, Jr., S. Hellman, S.A. Rosenberg (eds.), J.B. Lippincott Company, New York, pp. 682-701, 1991.

Goodman, L.A., Livingston, P.O. and Walkley, S.U.: Ectopic dendrites occur only on cortical pyramidal cells containing elevated GM2 ganglioside in  $\alpha$ -mannosidosis. Proc. Natl. Acad. Sci. USA 88: 11330-11334, 1991.

Livingston, P.O., Koganty, R., Longenecker, B.M., Lloyd, K.O. and Calves, M.: Studies on the immunogenicity of synthetic and natural Thomsen-Friedenreich (TF) antigens in mice: Augmentation of the response by Quil A and SAF-m adjuvants and analysis of the specificity of the responses. Vaccine Res. 1: 99-109, 1992.

Steffens, T.A. and Livingston, P.O. The status of adjuvant therapy of melanoma. IN: Surgical Oncology Clinics of North America. W. B. Saunders Company, Philadelphia, Pennsylvania, Volume 1, pp. 307-333, 1992.

O'Boyle, K.P., Zamore, R., Adluri, S., Cohen, A., Kemeny, N., Welt, S., Lloyd, K.O., Oettgen, H.F., Old, L.J. and Livingston, P.O. Immunization of colorectal cancer patients with modified ovine submaxillary gland mucin and adjuvants induces IgM and IgG antibodies to sialylated Tn. Cancer Res. 52: 5663-5667, 1992.

Livingston, P.O. Construction of cancer vaccines with carbohydrate and protein (peptide) tumor antigens. Current Opinion in Immunology 4: 624-629, 1992.

Hamilton, W.B., Helling, F., Lloyd, K.O. and Livingston, P.O. Ganglioside expression on human malignant melanoma assessed by quantitative immune thin layer chromatography. Int. J. Cancer 53: 566-573, 1993.

Livingston, P.O., Calves, M.J., Helling, F., Zollinger, W.D., Blake, M.S. and Lowell, G.H. GD3/proteosome vaccines induce consistent IgM antibodies against the ganglioside GD3. Vaccine 11, 1199-1204, 1993.

Ren, S., Scarsdale, J.N., Ariga, T., Zhang, Y., Slominski, A., Livingston, P.O., Ritter, G., Kushi, Y. and Yu, R.K. Characterization of a hamster melanoma-associated ganglioside antigen as 7-O-acetylated disialoganglioside GD3. J. Lipid Res. 34, 1565-1572, 1993.

Livingston, P.O. Conference Overview. IN: Specific Immunotherapy of Cancer with Vaccines. Annals of New York Academy of Sciences, J.C. Bystry, S. Ferrone, P.O. Livingston, (eds.), vol. 690, 1-5, 1993.

Livingston, P.O. Approaches to augmenting the IgG antibody response to melanoma ganglioside vaccines. IN: Specific Immunotherapy of Cancer with Vaccines. Annals of New York Academy of Sciences, J.C. Bystry, S. Ferrone, P.O. Livingston (eds.), vol. 690, 204-213, 1993.

Helling, F., Shang, Y., Calves, M., Oettgen, H.F. and Livingston, P.O. Increased immunogenicity of GD3 conjugate vaccines: Comparison of various carrier proteins and selection of GD3-KLH for further testing. Cancer Res. 54, 197-203, 1994.

Livingston, P.O., Wong, G.Y.C., Adluri, S., Tao, Y., Padavan, M., Parente, R., Hanlon, C., Calves, M.J., Helling, F., Ritter, G., Oettgen, H.F. and Old, L.J. Improved survival in AJCC stage III melanoma patients with GM2 antibodies: A randomized trial of adjuvant vaccination with GM2 ganglioside. J. Clin. Oncol., In Press.

Livingston, P.O., Adluri, S., Hughes, M.H., Calves, M.J., Raychaudhuri, S. and Merritt, J.A. A Phase I trial of the immunological adjuvant SAF-m in melanoma patients vaccinated with the anti-idiotypic antibody MELIMUNE<sup>TM</sup>-1. Vaccine Res., In Press.

Livingston, P.O. Development of generic vaccines for colorectal carcinoma. IN: Cancer of the Colon, Rectum and Anus, A.M. Cohen, S.J. Winawer (eds.), McGraw Hill Inc., New York, In Press, 1994.

Livingston, P.O., Adluri, S., Helling, F., Yao, T-J., Kensil, C.R., Newman, M.J. and Marciani, D. Phase I trial of immunological adjuvant QS-21 with a GM2 ganglioside-KLH conjugate vaccine in patients with malignant melanoma. Vaccine, In Press.

Helling, F., Zhang, A., Shang, A., Adluri, S., Calves, M., Koganty, R., Longenecker, B.M., Oettgen, H.F. and Livingston, P.O. GM2-KLH conjugate vaccine: Increased immunogenicity in melanoma patients after administration with immunological adjuvant QS-21. Submitted.

Adluri, S., Helling, F., Calves, M.J., Koganty, R., Longenecker, B.M., Lloyd, K.O. and Livingston, P.O. Immunogenicity of synthetic TF- and sTn-KLH conjugates in colorectal carcinoma patients. Submitted.



ABSTRACTS

Livingston, P.O., Bean, M.A., Oettgen, H.F. and Old, L.J.: Cytotoxicity (CTX) of melanoma patients' lymphocytes for autologous melanoma cells. Proc. Amer. Assoc. Cancer Res. 18: 95, 1977.

Livingston, P.O., Pinsky, C.M., Oettgen, H.F. and Old, L.J.: Antibody dependent cell mediated cytotoxicity for autologous melanoma. Proc. Amer. Assoc. Cancer Res. 19: 208, 1978.

Livingston, P.O., Shiku, H., Pinsky, C.M., Oettgen, H.F. and Old, L.J.: Effects of autologous melanoma cell vaccine on cell-mediated cytotoxicity for autologous melanoma cells. Proc. Amer. Assoc. Cancer Res. 20: 113, 1979.

Pinsky, C.M., Wittes, R.E., Livingston, P.O., Krown, S.E., Hirshaut, Y. and Oettgen, H.F.: Surgical adjuvant chemotherapy with or without pseudomonas vaccine in patient with stage II malignant melanoma. Proc. Amer. Assoc. Cancer Res. 21: 186, 1980.

Livingston, P.O., Takeyama, H., Houghton, A.N., Albino, A., Pinsky, C.M., Oettgen, H.F. and Old, L.J.: Serological response to vaccination with cultured allogeneic melanoma cells. Proc. Amer. Assoc. Cancer Res. 22: 283, 1981.

Kerr, D., Krown, S.E., Livingston, P.O., Pinsky, C.M., and Oettgen, H.F.: Treatment of melanoma skin metastases with intralesional DNCB. Proc. Amer. Assoc. Cancer Res. 22: 528, 1981.

Livingston, P.O., DeLeo, A.B., Oettgen, H.F. and Old, L.J.: Comparison of approaches for augmenting the serological response to the Meth A antigen. Cyclophosphamide is most effective. Proc. Amer. Assoc. Cancer Res. 24: 196, 1983.

Real, F.X., Mattes, M.J., Houghton, A.N., Livingston, P.O., Lloyd, K.O., Oettgen, H.F. and Old, L.J.: Unique (class I) tumor antigens of a human melanoma. Serological and biochemical studies. Proc. Amer. Assoc. Cancer Res. 24: 233, 1983.

Livingston, P.O., Hoffmann, M.K., Enker, W.E., Pinsky, C.M. and Oettgen, H.F.: Inhibition of suppressor cell activity in melanoma patients by cyclophosphamide. Amer. Soc. Clin. Onc. 25: 58, 1984.

Livingston, P.O., Jones, M., Oettgen, H.F. and Old, L.J.: Augmentation of the serological response to Meth A antigen by cyclophosphamide and endotoxin. Proc. Amer. Assoc. Cancer Res. 25: 279, 1984.

Livingston, P.O., Jones Calves, M. and Natoli, Jr., E.J.: Approaches to augmenting the immunogenicity of the ganglioside GM2 in mice. Proc. Amer. Assoc. Cancer Res. 27: 1445, 1986.

Natoli, Jr., E.J., Livingston, P.O., Cordon-Cardo, C., Pukel, C.S., Lloyd, K.O., Wiegandt, H., Szalay, J., Oettgen, H.F. and Old, L.J.: Murine monoclonal antibody detecting GM2, a ganglioside expressed by cells of neuroectodermal origin. Proc. Amer. Assoc. Cancer Res. 27: 1444, 1986.

Livingston, P.O., Jones Calves, M., Natoli, Jr., E.J.: Immunization of mice with the gangliosides GD3, GD2, GM3 and GM2. Proc. Amer. Assoc. Cancer Res. 28: 374, 1987.  
Livingston, P.O., Ritter, G., Srivastava, P., Padavan, M., Calves, M.J., Oettgen, H.F. and Old, L.J.: Characterization of IgG and IgM antibodies induced in melanoma

patients by immunization with purified GM2 ganglioside.  
Amer. Soc. Clin. Onc. 8: 289, 1989.

Ritter, G., Boosfeld, E., Yu, R.K., Oettgen, H.F., Old, L.J. and Livingston, P.O.: Development of melanoma vaccines: immunogenicity of O-acetyl GD3.  
Proc. Amer. Assoc. Cancer Res. 30: 384, 1989.

Ritter, G., Boosfeld, E., Calves, M.J., Oettgen, H.F., Old, L.J. and Livingston, P.O.: Immunogenicity of GD3 derivatives. Proc. Amer. Assoc. Cancer Res. 31: 279, 1990.

Ritter, G., Yu, R.K., Oettgen, H.F., Old, L.J. and Livingston, P.O.: Immunogenicity of 9-O-acetyl GD3 purified from bovine buttermilk in patients with melanoma. Proc. Amer. Assoc. Cancer Res. 31: 282, 1990.

O'Boyle, K., Cohen, A., Kemeny, N., Enker, W., Sigurdson, E., Welt, S., Lloyd, K.O., Oettgen, H.F., Old, L.J. and Livingston, P. Immunization of colon cancer patients with ovine sialomucin (OSM) containing Tn and sialylated Tn antigens. Amer. Soc. Clin. Onc. 9: 127, 1990.

Livingston, P.O., Koganty, R., Mackie, E., Longenecker, M. and Calves, M. The immunogenicity of synthetic Thomsen-Friedenreich (T) antigen is augmented by covalent attachment to KLH and the use of adjuvants. Proc. Amer. Assoc. Cancer Res. 32: 252, 1991.

O'Boyle, K.P., Zamore, R., Cohen, A., Welt, S., Longenecker, M., Lloyd, K.O., Old, L., Oettgen, H.F. and Livingston, P. Immunization of colorectal cancer patients with OSM vaccine elicits antibodies to sialylated Tn. Proc. Amer. Assoc. Cancer Res. 32: 256, 1991.

Livingston, P.O. Immunotherapy of melanoma and colon cancer with purified carbohydrate vaccines. Proc. Amer. Assoc. Cancer Res. 32: 491, 1991.

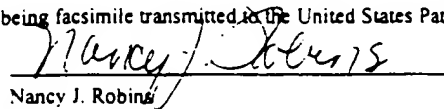
Helling, F., Lloyd, K.O., Oettgen, H.F. and Livingston, P.O. Increased immunogenicity of GD3 ganglioside after covalent attachment to proteins. Proc. Amer. Assoc. Cancer Res. 33: 335, 1992.

Hamilton, W.B., Helling, F., Lloyd, K.O. and Livingston, P.O. Ganglioside expression on human malignant melanoma detected by immune thin layer chromatography. Proc. Amer. Assoc. Cancer Res. 33: 335, 1992.

Livingston, P.O., Calves, M.J., Helling, F., Lowell, G.H., Zollinger, W.D. and Blake, M.S. Approaches to augmenting the immunogenicity of GD3 ganglioside in mice: Selection of proteosome vaccines for study in man. Proc. Amer. Assoc. Cancer Res. 33: 335, 1992.

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on November 4, 1996

  
Nancy J. Robins

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Lynn E. Spitler et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

**DECLARATION OF JEAN CLAUDE BYSTRYN, MD  
PURSUANT TO 37 C.F.R. § 1.132**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, Jean Claude Bystry, MD, declare as follows:

1. I am a member of the Department of Dermatology at the New York University Medical Center. A copy of my *Curriculum Vitae* is attached hereto as Exhibit A. I have no association or connection with Jenner Technologies, the assignee herein.

2. I have reviewed the Declaration Under 37 C.F.R. 1.132 prepared by Dr. Lynn E. Spitler describing the results of a clinical study directed to the use of prostate specific antigen (PSA) as an active ingredient in an antiprostata cancer vaccine. I am also familiar with the study itself. and with the results that were obtained.

3. The purpose of the study was to obtain evidence that the vaccines would raise a sufficient cellular immune response to have a beneficial effect with respect to prostate tumors. Such a result could be shown directly by measuring cytotoxic lymphocyte (CTL) generation; however, I am aware that this was not possible in these studies because the assay was not satisfactory because of the lack of an appropriate target cell for the assay.

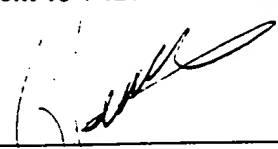
4. The responses measured are understood in the art to be satisfactory substitutes for measuring CTLs. Thus, the proliferation of lymphocytes from two of the patients in response to contact with PSA or in response to peptides representing putative PSA epitopes is indicative of an appropriate cellular immune response. The ability of PSA or PSA derived peptides to stimulate cytokine production -- i.e., gamma interferon and IL-4 production -- from lymphocytes in these patients also indicates that the cellular response is obtained specifically with respect to PSA. The observation of the development of a positive skin test response to PSA in one patient is also consistent with these observations showing the development of cell-mediated immunity in the patients.

5. In my opinion, the results obtained in this clinical study provide evidence that the vaccines are likely to be effective in exerting a beneficial effect on patients with prostate tumors or at risk for prostate tumors.

6. The efficacy shown for the vaccine tested in the foregoing clinical studies further provides evidence that analogous vaccines based on host tissue antigen, such as prostate specific membrane antigen (PSMA) and prostate acid phosphatase (PAP) would behave in a similar manner. It is also known that if the entire antigen is effective as a vaccine, portions of the antigen may be effective as well, especially if manipulated by art-known methods to enhance their immunogenicity, such as by coupling them to carrier.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

October 10, 1996

  
\_\_\_\_\_  
Jean Claude Bystryn, MD

10/30/86

# CURRICULUM VITAE

**JEAN-CLAUDE BYSTRYN, M.D.**

**DATE OF BIRTH:** May 8, 1938

**PLACE OF BIRTH:** Paris, France (U.S. Citizen)

## EDUCATION:

- 1958 B.S. University of Chicago (biochemistry), Special honors in the natural sciences
- 1962 M.D. New York University School of Medicine, New York, NY

## POST-GRADUATE TRAINING:

- 1962-1963 Intern (mixed), Montefiore Hospital, New York, NY
- 1963-1964 Residency (medicine), Montefiore Hospital, New York, NY
- 1966-1969 Residency (dermatology), NYU School of Medicine, New York, NY
- 1968-1969 USPH post-doctoral research fellow (vascular physiology), Laboratory of Dr. Chester Hyman, Dept. of Physiology and Dermatology, University of Southern California, Los Angeles, CA
- 1969-1972 USPH post-doctoral research fellow (immunology), Laboratory of Dr. Jonathan Uhr, Dept. of Medicine, NYU School of Medicine, New York, NY

## MEDICAL LICENSURE:

- 1963 State of New York
- 1964 State of California

## BOARD CERTIFICATION:

- 1970 American Board of Dermatology
- 1986 American Board of Immunodermatopathology

## MILITARY EXPERIENCE:

- 1964-1966 USPHS Heart Disease Control Officer, Albany, NY

## HOSPITAL AND TEACHING APPOINTMENTS:

- 1969- Assistant Visiting Dermatologist, Bellevue Hospital Center, New York
- 1969- Assistant Attending, University Hospital, NYU School of Medicine, New York
- 1970-1971 Instructor, Dept. of Dermatology, NYU School of Medicine, New York
- 1970-1997 Associate Attending, Depts. of Dermatology and Syphilology, Bellevue Hospital Center, New York
- 1971-1972 Assistant Professor of Clinical Dermatology, Dept. of Dermatology, NYU School of Medicine, New York
- 1972-1978 Assistant Professor, Dept. of Dermatology, NYU School of Medicine, New York
- 1972- Director, Immunofluorescence Laboratory, Dept. of Dermatology, NYU School of Medicine, New York
- 1974- Co-Director, Bullous Disease Clinic, Skin and Cancer Unit, NYU School of Medicine, New York
- 1978-1984 Associate Professor, Dept. of Dermatology, NYU School of Medicine, New York
- 1982- Director, Melanoma Immunotherapy Clinic, Skin and Cancer Unit, NYU Medical Center, New York
- 1983-1992 Executive Committee, Kaplan Cancer Center, NYU Medical Center, New York
- 1983- Director, Melanoma Program, Kaplan Cancer Center, NYU Medical Center, New York
- 1984- Professor, Dept. of Dermatology, NYU School of Medicine, New York

*Dr. hinh A*

**BYSTRYN, JEAN-CLAUDE**  
*curriculum vitae*

page - 2 -

#### **HONORS AND AWARDS:**

1954 Ford Foundation Fellowship for early entrance to colleg  
 1968 Husik Prize in Dermatology, NYU School of Medicin  
 1979 Irma T. Hirschl Career Scientist Award  
 1985 Skin Cancer Foundation Annual Award  
 1987 Philippine Society of Dermatology, Honorary Member  
 1990 AOA  
 1990 Society Francaise de Dermatologie, Honorary Member  
 1991 The Hellenic Society of Dermatology, Honorary Member

#### **CHAIRMAN OF SYMPOSIA/WORKSHOPS AT NATIONAL AND INTERNATIONAL CONFERENCES:**

1982 Int'l Symposium on Stratum Corneum, Cardiff  
 1982 Fifth Southeast Asian Conference on Dermatology, Manila  
 1982 XVIth Int'l Congress on Dermatology, Tokyo  
 1983 Joint Annual Meeting of the Society of Investigative Dermatology and European Society for  
 Dermatologic Research, Washington, DC  
 1983 First World Congress on Cancers of the Skin, New York  
 1983 XIIth Int'l Pigment Cell Conference  
 1985 Second World Congress on Cancer of the Skin, New York  
 1985 Joint International Meeting of the SID and JCID, Washington, DC  
 1987 XVIth International Congress of Dermatology, Berlin  
 1988 Second International Conference on Melanoma, Venice  
 1990 NIH Research Workshop on Alopecia Areata, Organizer  
 1992 XVIIth International Congress of Dermatology, New York  
 1993 Third International Conference on Melanoma, Venice  
 1993 New York Academy of Sciences Conference on "Specific Immunotherapy of Cancer with Vaccines,"  
 Washington, D.C., Conference Chair  
 1994 International Symposium "Skin Therapy Update '94," Crete, Co-Chairman  
 1995 Sixth World Congress of Cancers of the Skin, Buenos Aires, Chairperson of Plenary Session on Basic  
 Science  
 1996 International Conference for Apheresis 1996, Kyoto, Chair

#### **PROFESSIONAL SOCIETIES:**

Society Francaise de Dermatologie, Honorary Member  
 Philippine Society of Dermatology, Honorary Member  
 American Academy of Dermatology  
 American Dermatological Association  
 American Association of Immunologists  
 American Association for Cancer Research  
 Society for Investigative Dermatology  
 American Society for Cell Biology  
 International Society for Tropical Dermatology  
 American Federation for Clinical Research  
 New York Dermatological Society  
 Dermatology Foundation  
 International Pigment Cell Society  
 PANAMERICAN Society for Pigment Cell Research  
 International Society for Vaccines  
 Clinical Immunology Society  
 Society for Biological Therapy

**BYSTRYN, JEAN-CLAUDE**  
curriculum vitae

page - 3 -

# **ASSIGNMENTS IN PROFESSIONAL SOCIETIES OR MEDICAL JOURNALS:**

- 1975-1978 Mycosis Fungoides Cooperative Group, Chairman, Immunotherapy Panel
- 1980-1982 Dystrophic Epidermolysis Bullosa Foundation, Board of Medical Advisors
- 1980- Skin Cancer Foundation, Board of Medical Advisors and Chairman Grant Review Committee
- 1981-1980 American Academy of Dermatology, Task Force on Immunopathology
- 1986-1987 New York Dermatological Society, Secretary
- 1987-1988 New York Dermatological Society, President
- 1983- National Alopecia Areata Foundation, Medical Advisory Board
- 1987- Molecular Therapeutic, Editorial Board
- 1990- Vaccine Research, Editorial Board
- 1992-1998 Journal of the European Academy of Dermatology and Venereology, Editorial Board
- 1993- Society for Investigative Dermatology, Committee for Industrial Sponsorship
- 1993- American Dermatological Association, Membership Committee
- 1993- National Vitiligo Foundation, Medical Advisory Board
- 1994- American Academy of Dermatology, Commission on Melanoma
- 1994- American Board of Dermatology, Training Program in Clinical and Laboratory Dermatological Immunology Review Committee
- 1994- American Board of Dermatology, Recertification Committee
- 1995- American Skin Association, Medical/Scientific/Policy Advisory Committee
- 1998 NIH Ad Hoc Reviewer, Biological Response Modifier Program
- 1998-2000 American Academy of Dermatology, Manpower Committee

## **PUBLICATIONS**

1. BYSTRYN J-C, HYMAN C. Skin blood flow in atopic dermatitis. *J Invest Derm* 52:189, 1969.
2. BYSTRYN J-C, FREEDMAN RI, HYMAN C. Clearance of iodoantipyrene from Mecholyl blanched skin in atopics. *Arch Derm* 100:165, 1969.
3. LEVAN NE, BYSTRYN J-C, HYMAN C. Temperature and blood flow in macules of lepromatous leprosy. *Int J Leprosy* 37:249, 1969.
4. BYSTRYN J-C, GRAF MW, UHR JW. Regulation of antibody formation by serum antibody. II. Removal of specific antibody by means of exchange transfusion. *J Exp Med* 132:1279, 1970.
5. UHR JW, BYSTRYN J-C, GRAF MW. The regulation of antibody formation. In: Morphological and Fundamental Aspects of Immunity, ed. by Lindahl-Kiessling, Alm and Hanna; Plenum Press, New York, 1971, pp 395.
6. BYSTRYN J-C, FELLNER M. Erythema multiforme. In: Immunological Diseases, 2nd edition, ed. by Samter; Little, Brown and Co., Boston, MA, 1971.
7. SCHENKEIN I, BYSTRYN J-C, UHR JW. Specific removal of in vivo antibody by extracorporeal circulation of an immunoadsorbent in gel. *J Clin Invest* 50:1884, 1971.
8. BYSTRYN J-C, SCHENKEIN I, UHR JW. A model for the regulation of antibody synthesis by serum antibody. In: Progress in Immunology, vol. 1, ed. by B Amos; Academic Press, New York, 1971, pp 627.
9. BYSTRYN J-C. Drug fever. Am J Med Science 264:467, 1972.
10. BYSTRYN J-C, BART RS, LIVINGSTON P, KOPF AW. Immunity to murine melanoma. *J Invest Derm* 60:249, 1973.
11. BYSTRYN J-C, SISKIND GW, UHR JW. Binding of antigen by immunocytes 1. Effect of ligand valency on binding affinity of MOPC 315 cells for DNP-conjugates. *J Exp Med* 137:301, 1973.



**BYSTRYN, JEAN-CLAUDE**  
*curriculum vitae*

Page - 4 -

12. **BYSTRYN J-C, ABEL E, WEIDMAN A.** Antibodies against the cytoplasm of human epidermal cells. Arch Derm 108:241-244, 1973.
13. **UHR JW, SCHENKEIN I, BYSTRYN J-C.** Immunoregulation. In: Specific Receptors of Antibodies, Antigens, and Cells. (Third Int'l Convocation of Immunology, Buffalo, NY) S. Karger, Basel, 1973, pp 367.
14. **BYSTRYN J-C, SCHENKEIN I, UHR JW.** Double antibody radioimmunoassay for tumor antibodies. J Natl Cancer Inst 52:911-915, 1974.
15. **BYSTRYN J-C, SCHENKEIN I, BAUR S, UHR JW.** Partial isolation and characterization of antigen(s) associated with murine melanoma. J Natl Cancer Inst 52:1263-1269, 1974.
16. **BYSTRYN J-C, BART RS, LIVINGSTON P, KOPF AW.** Growth and immunogenicity of B16 murine melanoma. J Invest Derm 63:369-373, 1974.
17. **BYSTRYN J-C, ABEL E, DEFEO C.** Pemphigus foliaceus: subcorneal intercellular antibodies of unique specificity. Arch Derm 110:857-862, 1974.
18. **ROBINS DN, DEFEO C, BYSTRYN J-C.** Hepatotoxicity of azathioprine. Cutis 14:600, 1974.
19. **BYSTRYN J-C, SISKIND GW, UHR JW.** Binding of antigen by immunocytes. II. Effect of specific Ig on binding of antigen by MOPC 315 cells. J Exp Med 141:1227, 1975.
20. **BYSTRYN J-C.** Nature of antigens associated with murine melanoma. In: Pigment Cell, ed. by V Riley, S Karger, Basel, 2:158, 1976.
21. **ABEL EA, BYSTRYN J-C.** Epidermal cytoplasmic antibodies: incidence and type in normal persons and patients with melanoma. J Invest Derm 66:44-48, 1976.
22. **ABEL EA, BYSTRYN J-C.** Reproducibility of the immunofluorescent test for antimelanoma antibodies. J Invest Derm 66:117, 1976.
23. **BYSTRYN J-C.** Release of tumor associated antigens by murine melanoma cells. J Immunol 116:1302, 1976.
24. **BYSTRYN J-C.** Epidermal antigens. Int J Derm 16:645-656, 1977.
25. **BYSTRYN J-C, RODRIGUEZ J.** Pemphigus foliaceus associated with absence of intercellular antigens in the lower layers of the epidermis. Arch Derm 113:1696, 1978.
26. **BYSTRYN J-C.** Clinical significance of basal cell layer antibodies. Arch Derm 113:1380-1382, 1977.
27. **BYSTRYN J-C.** Release of cell-surface tumor-associated antigens by viable human melanoma cells. J Natl Cancer Inst 59:325, 1977.
28. **BYSTRYN J-C, SMALLEY JR.** Identification and solubilization of iodinated cell-surface human melanoma associated antigens. Int J Cancer 20:165, 1977.
29. **BYSTRYN J-C, NASH M, ROBINS P.** Epidermal cytoplasmic antigens: II. concurrent presence of antigens of different specificities in normal human skin. J Invest Derm 71:110-113, 1978.
30. **BYSTRYN J-C, RODRIGUEZ J.** Absence of intercellular antigens in the deep layers of the epidermis in pemphigus foliaceus. J Clin Invest 61:339-348, 1978.
31. **BYSTRYN J-C.** Antibody response and tumor growth in syngeneic mice immunized to partially purified B16 melanoma associated antigens. J Immunol 120:96-101, 1978.
32. **LIM HW, BYSTRYN J-C.** Bullous disease. Clin Obst & Gynecol 21:1007, 1978.

**BYSTRYN, JEAN-CLAUDE**  
**Curticulus vitae**

page - 3 -

33. KLAUS SN, LERNER AB, BYSTRYN J-C, MOELLMANN G, QUEVEDO W, SOBER AJ: IX. Malignant melanoma and vitiligo. J Invest Dermatol 1979;73 (No 5, pt II):461-64.
34. AUERBACK R, BYSTRYN J-C. Effect of plasmapheresis and immunosuppressive therapy of intercellular antibodies in pemphigus vulgaris. Arch Derm 115:728-730, 1979.
35. BYSTRYN J-C, FRANCES C. Cytoplasmic differentiation antigens of human epidermal cells. Transplantation 27:392-396, 1979.
36. BYSTRYN J-C. Double antibody antigen binding assay for antibodies to melanoma. In: Immunodiagnosis of Human Cancer, ed. by RB Herberman; Elsevier/North Holland, New York, pp 581, 1979.
37. BYSTRYN J-C, ORENTREICH N, STENGEL F. Direct immunofluorescence studies in alopecia areata and male pattern alopecia. J Invest Derm 73:317, 1979.
38. BYSTRYN J-C, SMALLEY JR. Shedding of tumor-associated antigens by viable human malignant melanoma cells. In: Pigment Cell, ed. by SN Klaus, S Karger, Basel, 5:155, 1979.
39. BYSTRYN J-C. Immunofluorescence studies in transient acantholytic dermatosis. Am J Dermatopath 1:325-327, 1979.
40. BHAVANADANVP, KEMPER J, BYSTRYN J-C. Purification and partial characterization of a murine melanoma-associated antigen. J Biol Chem 255:5145-5143, 1980.
41. BYSTRYN J-C. The Immunology of human malignant melanoma. Int J Derm 19:375-378, 1980.
42. ENG K, RUOFF J, BYSTRYN J-C. Behcet's syndrome: an unusual cause of colonic ulceration and perforation. Am J Gastroenterology 75:57-59, 1981.
43. BYSTRYN J-C, TEDHOLM CA, HEANEY-KERAS J. Release of surface macromolecules by human melanoma and normal cells. Cancer Res 41:910-914, 1981.
44. NORDLUND J, HOWANITZ N, BYSTRYN J-C, FORGET BM, LERNER A. Antipigment cell factors and mucocutaneous candidiasis. Arch Derm 117:210-212, 1981.
45. WOLF D, BYSTRYN J-C. Alterations in antigenic properties of normal epidermis adjacent to cutaneous malignancies. J Invest Derm 76:442-444, 1981.
46. FAURE M, EISINGER M, BYSTRYN J-C. Decreased expression of epidermal cytoplasmic antigens in cultured human keratinocytes. J Invest Derm 76:347-351, 1981.
47. HOWANITZ N, NORDLUND JJ, LERNER AB, BYSTRYN J-C. Antibodies to melanocytes in patients with vitiligo and chronic mucocutaneous candidiasis. Arch Derm 117:705-708, 1981.
48. FAURE M, EISINGER M, BYSTRYN J-C. Pemphigus, bullous pemphigoid and upper-cytoplasmic antigens: changes in expression in human skin cultures. Arch Derm Res 271:73-82, 1981.
49. BYSTRYN J-C, HOWANITZ N, NORDLUND JJ, LERNER AB. Antibodies to melanocytes in patients with vitiligo. In: Pigment Cell 1981, Phenotypic Expression in Pigment Cells, ed. by M Seiji; University of Tokyo Press, Tokyo, pp 463-466, 1981.
50. BYSTRYN J-C. Intercellular deposits of complement in skin of patients with pemphigus. Arch Derm 117:682, 1981.
51. BYSTRYN J-C. Skin tumor markers. In: Human Cancer Markers, ed. by S Sell and B Wahren; Humana Press, Clifton, NJ 2:89-104, 1982.

**BYSTRYN, JEAN-CLAUDE**  
*curriculum vitae*

Page - 6 -

52. **BYSTRYN J-C, PERLSTEIN J.** Autocatabolism of surface macromolecules shed by human melanoma cells. *Cancer Res* 42:2232-2237, 1982.
53. **BOCTOR AM, BYSTRYN J-C.** Degradation of tumor associated antigens shed by human melanoma cells in culture. *Cancer Res* 42:2121-2125, 1982.
54. **HEANEY-KIERAS J, BYSTRYN J-C.** Identification and purification of a 75k dalton cell-surface human melanoma associated antigen. *Cancer Res* 42:2310-2319, 1982.
55. **BYSTRYN J-C, JACOBSEN JS, LIU P, HEANEY-KIERAS J.** Comparison of cell-surface human melanoma associated antigens identified by rabbit and murine antibodies. *Hybridoma* 1:465-472, 1982.
56. **BYSTRYN J-C.** Shedding and degradation of cell-surface macromolecules and tumor associated antigens by human melanoma. In: Melanoma Antigens and Antibodies, ed. by RA Reisfeld and S Ferrone; Plenum Press, New York, pp 37-52, 1982.
57. **ZHU X-J, BYSTRYN J-C.** Heterogeneity of pemphigoid antigens. *J Invest Derm* 80:16-20, 1983.
58. **BYSTRYN J-C.** Natural stratum corneum antibodies and antigens. In: Stratum Corneum, ed. by R Marks and G Plewig; Springer-Verlag, Berlin, pp 112-126, 1983.
59. **NAUGHTON GK, EISINGER M, BYSTRYN J-C.** Antibodies to normal human melanocytes in vitiligo. *J Exp Med* 158:246-251, 1983.
60. **JACOBS M, SCHNED ES, BYSTRYN J-C.** Variability of the lupus band test: results in 18 patients with systemic lupus erythematosus. *Arch Derm* 119:863-869, 1983.
61. **MEDINA AL, ROBBINS TD, BYSTRYN J-C., ACKERMAN AB.** Limitations in the diagnosis of vesiculo-bullous diseases. *Am J Dermatopath* 5(1):7-11, 1983.
62. **NAUGHTON GK, EISINGER M, BYSTRYN J-C.** Detection of antibodies to melanocytes in vitiligo by specific immunoprecipitation. *J Invest Derm* 81:540-542, 1983.
63. **GOLDBERG D, SABOLINSKI M, BYSTRYN J-C.** Regional variations in the expression of bullous pemphigoid antigen in human skin. *J Invest Derm* 82:326-328, 1984.
64. **JOHNSTON D, BYSTRYN J-C.** Mechanism of autodegradation of cell surface macromolecules shed by human melanoma cells. *Exp Cell Res* 152:179-187, 1984.
65. **BYSTRYN J-C.** Adjuvant therapy of pemphigus. *Arch Derm* 120:941-951, 1984.
66. **ZHU X-J, EISINGER M, BYSTRYN J-C.** Differentiation and shedding of surface macromolecules of human keratinocytes. *J Invest Derm* 83:340-343, 1984.
67. **NAUGHTON GK, LIPKIN G, BYSTRYN J-C.** Expression of vitiligo antigen on a revertant line of hamster melanoma cells. *J Invest Derm* 83:317-319, 1984.
68. **BYSTRYN J-C.** Immunology and immunotherapy of human malignant melanoma. *Dermatologic Clinics* 3:327-334, 1985.
69. **JOHNSTON D, BYSTRYN J-C.** Effect of tunicamycin on release of macromolecules and tumor antigens by human melanoma cells. *Cancer Res* 45:1772-1777, 1985.
70. **GOLDBERG DJ, SABOLINSKI M, BYSTRYN J-C.** Bullous pemphigoid antibodies. Human skin as a substrate for indirect immunofluorescence assay. *Arch Derm* 121:1137-1140, 1985.

**BYSTRYN, JEAN-CLAUDE**  
curriculum vitae

Page - 7 -

71. **BYSTRYN J-C, NAUGHTON GK.** The significance of vitiligo antibodies. *The Journal of Dermatology* 12:1-9, 1985.
72. **BYSTRYN J-C, BERNSTEIN P, LIU P, VALENTINE F.** Immunophenotyp of human melanoma cells in different metastases. *Cancer Res* 45:5603-5607, 1985.
73. **LIPKIN G, NAUGHTON GK, ROSENBERG M, BYSTRYN J-C.** Vitiligo-related pigment cell differentiation antigens are expressed on malignant melanoma cells following phenotypic reversion induced by contact inhibitory factor. *Differentiation* 30:35-39, 1985.
74. **BYSTRYN J-C.** Interpretation of immunofluorescence tests in dermatology. *Progress in Dermatology* 10:1-8, 1985.
75. **BYSTRYN J-C, VALENTINE F, BERNSTEIN P.** Tumor antigen heterogeneity and the specific immunotherapy of melanoma. In: Biological Molecular and clinical Aspects of Pigmentation, ed. by J Bagnana, SW Klaus, E Paul and M Schantl; Univ. of Tokyo Press, Tokyo, pp 391-396, 1985.
76. **HEANEY-KIERAS J, BYSTRYN J-C.** Identification and purification of 115kd and 125kd cell-surface human melanoma-associated antigens. *J Nat Cancer Inst* 77:643-648, 1986.
77. **NAUGHTON GK, MAHAFFEY M, BYSTRYN J-C.** Antibodies to surface antigens of pigmented cells in animals with vitiligo. *Proc Soc Exp Biol and Med* 181:423-426, 1986.
78. **SISON-PONACIER L, BYSTRYN J-C.** Regional variations in antigenic properties of skin: A possible cause for disease-specific distribution of skin lesions. *J Exp Med* 164:2125-2130, 1986.
79. **BYSTRYN J-C, JACOBSEN S, HARRIS M, ROSES D, SPEYER J, LEVIN M.** Preparation and characterization of a polyvalent human melanoma antigen vaccine. *J Biol Resp Modif* 5:211-224, 1986.
80. **NAUGHTON GK, REGGIARDO D, BYSTRYN J-C.** Correlation between vitiligo antibodies and extent of depigmentation in vitiligo. *J Am Acad Derm* 15:978-981, 1986.
81. **BYSTRYN J-C, SABOLINSKI M.** Effect of substrate on indirect immunofluorescence tests for intercellular and basement membrane zone antibodies. *J Am Acad Derm* 15:973-977, 1986.
82. **BYSTRYN J-C, KOLKS-NAUGHTON G, LIPKIN G.** Vitiligo antigens: Relation to pigment cell differentiation and melanoma. In: Cutaneous Melanoma Status of Knowledge and Future Perspective, ed. by U Veronesi, N Cascinelli, M Santinami; Academic Press, London pp 191-197, 1987.
83. **JOHNSTON DJ, SCHACHNE J, BYSTRYN J-C.** Identification of immunogenic B16 melanoma-associated antigens. *J Biol Resp Modif* 6:109-120, 1987.
84. **BYSTRYN J-C, RIGEL D, FRIEDMAN RJ, KOPF A.** The prognostic significance of hypopigmentation in malignant melanoma. *Arch of Derm* 123:1053-1055, 1987.
85. **SISON-PONACIER L, BYSTRYN J-C.** Heterogenicity of pemphigus vulgaris antigens. *Arch Derm* 123:1507-1510, 1987.
86. **SABOLINSKI ML, BEUTNER EH, KRASNY S, KUMAR V, HUANG J, CHORZELSKI TP, SAMPAIO S, BYSTRYN J-C.** Substrate specificity of anti-epithelial antibodies of pemphigus vulgaris and pemphigus foliaceus sera in immunofluorescence tests on monkey and guinea pig esophagus sections. *J Invest Derm* 88:545-9, 1987.
87. **LIPKIN G, NAUGHTON GK, ROSENBERG M, BYSTRYN J-C.** Reversion of the malignant melanoma phenotype with induction of pigment cell differentiation antigens and in vitro growth control by a contact inhibitory factor.
88. **BONPA E, BYSTRYN J-C, ELKON KB.** Detection of immunoglobulin G antibodies in melanoma sera reactive with intercellular proteins. *J Invest Derm* 90:207-212, 1988.

**BYSTRYN, JEAN-CLAUDE**  
*cuticularis villosa*

Page - 8 -

89. BYSTRYN J-C, ORATZ R, HARRIS MN, ROSES DF, GOLOMB FM, SPEYER JL. Immunogenicity of a polyvalent melanoma antigen vaccine in humans. *Cancer* 61:1065-1070, 1988.
90. BYSTRYN J-C, PFEFFER S. Vitiligo and antibodies to melanocytes. In: Advances in Pigment Cell Research, ed. by Alan R. Liss, Inc., New York, New York. pp 195-208, 1988.
91. NORRIS DA, KISSINGER RM, NAUGHTON GM, BYSTRYN J-C. Evidence for immunologic mechanisms in human vitiligo: Patient's sera induce damage to human melanocytes in vitro by complement-mediated damage and antibody dependent cellular cytotoxicity (ADCC). *J Invest Derm* 90:783-789, 1988.
92. BYSTRYN J-C. Melanoma antigen vaccine. In: Proceedings of the XVIth World Congress of Dermatology, ed. by CE Ortanoe, R Stadler, and H Gollnick; Springer-Verlag, Berlin pp 681-682, 1988.
93. BYSTRYN J-C. The therapy of pemphigus. *Seminars in Dermatology* 7:186-194, 1988.
94. BYSTRYN J-C. Guest Editor. Diagnosis and management of bullous diseases. *Seminars in Dermatology* Vol. 7, 1988.
95. NORRIS DA, CAPIN L, MUGLIA JJ, OSBORN RL, ZERBIE GO, BYSTRYN J-C, TONNESEN MG. Enhanced susceptibility of melanocytes to different immunologic effector mechanisms in vitro: Potential mechanisms for postinflammatory hypopigmentation and vitiligo. *Pigment Cell Res, Supp* 1:113-123, 1988.
96. BYSTRYN J-C, DUGAN M, ORATZ R, SPEYER JL, HARRIS MN, ROSES DF. Vaccine immunotherapy of human malignant melanoma: relationship between method of immunization, immunogenicity, and tumor progression (1). In: Human Tumor Antigens and Specific Tumor Therapy, ed. by C. Fred Fox; Alan R. Liss, Inc., New York, pp 307-315, 1989.
97. BYSTRYN J-C. Immune mechanisms in vitiligo. In: Immune Mechanisms in Cutaneous Diseases, ed. by David A. Norris; Marcel Dekker, Inc., New York, and Basel, pp 447-473, 1989.
98. BYSTRYN J-C. Immunosurveillance and melanoma. *J Invest Derm* 92:318S-320S, 1989.
99. JOHNSTON D, BYSTRYN J-C. Immunogenicity and tumor protective activity of B16 melanoma vaccines. *Mol Biother* 1:218-222, 1989.
100. ORATZ R, COCKERALL C, SPEYER JL, HARRIS MN, ROSES DF, BYSTRYN J-C. Induction of tumor-infiltrating lymphocytes in malignant melanoma metastases by immunization to melanoma antigen vaccine. *J Biol Res Modif* 8:355-358, 1989.
101. BYSTRYN J-C. Autoimmune diseases of the skin. *Allergy Proceedings* 10:6:393-398, 1989.
102. BYSTRYN J-C. Serum antibodies in vitiligo patients. *Clin in Derm* 7:136-145, 1989.
103. TAN-LIM R, BYSTRYN J-C. Effect of plasmapheresis therapy on circulating levels of pemphigus antibodies. *J Am Acad Derm* 22:35-39, 1990.
104. BYSTRYN J-C. Vaccine immunotherapy of melanoma. In: Human Melanoma: From Basic Research to Clinical Application, ed. by S Ferrone; Springer-Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong, pp 513-530, 1990.
105. ZHU X-J, NIIMI Y, BYSTRYN J-C. Epidermolysis bullosa acquisita. *Arch Derm* 126:171-74, 1990.
106. DUGAN M, ORATZ R, BYSTRYN J-C. Immunotherapy in the treatment of malignant melanoma. In: Cancer of the Skin, ed. by RJ Friedman, DS Rigel, AW Kopf, MN Harris, D Baku; W.B. Saunders, pp. 569-579, 1990.
107. ZHU X-J, NIIMI Y, BYSTRYN J-C. Identification of a 160 kD molecule as a component of the basement membrane zone and as a minor bullous pemphigoid antigen. *J Invest Derm* 94:817-821, 1990.

**BYSTRYN, JEAN-CLAUDE**  
*curriculum vita*

page - 9 -

108. **BYSTRYN J-C.** Tumor vaccines. *Cancer and Metastasis Reviews*. 9:81-91, 1990.
109. **BYSTRYN J-C.** Shed melanoma antigens as vaccines. In: Biological Agents in the Treatment of Cancer, ed. by Peter Hersey, Newcastl , Australia, pp. 137-146, 1990.
110. **BYSTRYN J-C, HERSEY P.** Anti-melanoma vaccines. In: Cutaneous Melanoma, Biology and Management, ed. by N Cascinelli, Mario Santinami and U Veronesi, Masson spa, pp. 181-191, 1990.
111. **ZHU X-J, NIIMI Y, BYSTRYN J-C.** Molecular identification of major and minor bullous pemphigoid antigens. *J Am Acad Derm* 23:876-880, 1990.
112. **BYSTRYN J-C.** Vaccine treatment of melanoma. *The Skin Cancer Foundation Journal*, Vol. 9:39,62, 1991.
113. **ROSES DF, KARP NS, ORATZ R, DUBIN N, HARRIS MN, SPEYER J, BOYD A, GOLOMB FM, RUNSOHOFF J, DUGAN M, VALENTINE FT, COOPER J, BYSTRYN J-C.** Survival with regional and distant metastases from cutaneous malignant melanoma. *Surgery, Gynecology & Obstetrics*, 172:262-266, 1991.
114. **TAMESIS J, BYSTRYN J-C.** Immunologic aspects of hair loss. *J of Invest Derm* 96:88S, 1991.
115. **BYSTRYN J-C, ORATZ R.** Malignant melanoma. In: Dermatologic Diseases of the Skin, ed. by Robert Jordan; Appleton and Lange, pp. 539-551, 1991.
116. **IOANNIDES D, HYTIROGLOU P, PHELPS RG, BYSTRYN J-C.** Regional variation in the expression of pemphigus foliaceus, pemphigus erythematosus, and pemphigus vulgaris antigens in human skin. *J Invest Derm* 96:159-161, 1991.
117. **JOHNSTON D, BYSTRYN J-C.** Effect of cell wall skeleton and monophosphoryl lipid A adjuvant on the immunogenicity of a murine B16 melanoma vaccine. *J of the National Cancer Institute* 83:1240-1245, 1991.
118. **NIIMI Y, ZHU X-J, BYSTRYN J-C.** Identification of basement membrane zone antigens defined by antibodies that react to both the dermal and epidermal side of 1M sodium chloride split skin. *J of Invest Derm* 97:312-317, 1991.
119. **ORATZ R, DUGAN M, ROSES DF, HARRIS MN, SPEYER JL, HOCHSTER H, WEISSMAN J, HENN M, BYSTRYN J-C.** Lack of effect of cyclophosphamide on the immunogenicity of a melanoma antigen vaccine. *Cancer Res* 51:3643-3647, 1991.
120. **BYSTRYN J-C.** Immune response to melanoma. "The Melanoma Letter." 9(3):1-4, 1991.
121. **ORATZ R, BYSTRYN J-C.** Immunotherapy of malignant melanoma. In: Dermatologic Clinics, ed. by D Rigel; W.B. Saunders Company, pp. 669-682, 1991.
122. **HARNING R, CUI J, BYSTRYN J-C.** Relation between the incidence and level of pigment cell antibodies and disease activity in vitiligo. *J Invest Derm* 97:1076-1080, 1991.
123. **HARNING R, MAINOLFI E, BYSTRYN J-C, HENN M, MERLUZZI VJ, ROTHLEIN R.** Serum levels of circulating intercellular adhesion molecule-1 (ICAM-1) in human malignant melanoma. *Cancer Res* 51(18):5003-5, 1991.
124. **NIIMI Y, ZHU X-J, BYSTRYN J-C.** Identification of cicatricial pemphigoid antigens. *Arch Dermatol* 128:54-57, 1992.
125. **BYSTRYN J-C.** Limitations of direct immunofluorescence. *Arch Dermatol* 128(2):271-2, 1992.
126. **CUI J, BYSTRYN J-C.** A new europium release assay for complement-mediated cytotoxicity. *J of Immunological Methods*. 147:13-19, 1992.

**BYSTRYN, JEAN-CLAUDE**  
*curriculum vitae*

page - 10 -

127. **BYSTRYN J-C, ORATZ R, ROSES D, HARRIS M, HENN M, LEW R.** Relation between immune response to melanoma vaccine immunization and clinical outcome in stage II malignant melanoma. *Cancer* 69:1157-1164, 1992.
128. **IOANNIDES D, HYTIROGLOU P, PHELPS RG, BYSTRYN J-C.** Regional variation in the expression of epidermolysis bullosa acquisita antigen. *European J of Derm* 2:185-188, 1992.
129. **JOHNSTON D, ORLOW SJ, LEVY E, BYSTRYN, J-C.** Induction of B16 melanoma melanogenesis by a serum-free synthetic medium. *Experimental Cell Biology* 201:81-86, 1992.
130. **BYSTRYN J-C.** Polyvalent melanoma antigen vaccine. In: Cutaneous Melanoma: Clinical Management and Treatment Results Worldwide, ed. by C Balch, A Houghton, A Sober, G Milton; JB Lippincott, pp. 547-548, 1992.
131. **BYSTRYN J-C, HENN M, LI J, SHROBA S.** Identification of immunogenic human melanoma antigens in a polyvalent melanoma vaccine. *Cancer Res* 52:5948-5953, 1992.
132. **IOANNIDES D, BYSTRYN J-C.** Immunofluorescence abnormalities in lichen planopilaris. *Arch Dermatol* 128:214-218, 1992.
133. **CUI J, HARNING R, HENN M, BYSTRYN J-C.** Identification of pigment cell antigens defined by vitiligo antibodies. *J of Invest Derm* 98(2):182-5, 1992.
134. **BYSTRYN J-C.** Melanoma vaccines. *Int'l J of Clinical and Laboratory Research*. - 1992.
135. **BYSTRYN J-C, CUI J.** Vitiligo. In: The Molecular Pathology of Autoimmune Diseases, ed. by C.A. Bona, K.A. Siminovitch, M. Zanetti, A.N. Theofilopoulos; Harwood Academic Publishers, pp. 555-561, 1993.
136. **JOHNSTON D, BYSTRYN J-C.** Effect of the adjuvant SAF-M on the immunogenicity of a murine B16 melanoma vaccine. *Vaccine Res* 2(1):55-64, 1993.
137. **BYSTRYN J-C, HODAKE, GAO S-Q, CHUBA JV, AMOROSI EL.** A paraneoplastic mixed bullous skin disease associated with anti-skin antibodies and a B-cell lymphoma. *Arch Dermatol* 129:870-875, 1993.
138. **PION I, BYSTRYN J-C.** Expression of toxic epidermal necrolysis in grafted skin is donor site dominant. *Arch Dermatol* 129:1058-1059, 1993.
139. **CUI J, ARITA Y, BYSTRYN J-C.** Cytolytic antibodies to melanocytes in vitiligo. *J Invest Derm* 100:812-815, 1993.
140. **IOANNIDES D, BYSTRYN J-C.** Association of tissue-fixed cytoplasmic deposits of Ig in epidermal keratinocytes with lupus erythematosus. *Arch Dermatol* 129:1130-1135, 1993.
141. **BYSTRYN J-C.** Immunogenicity and clinical activity of a polyvalent melanoma antigen vaccine prepared from shed antigens. In: Specific Immunotherapy of Cancer with Vaccines, ed by J-C Bystryn, S Ferrone, P Livingston; Ann NY Acad Sci 690:190-203, 1993.
142. **BIELORY L, KUPERSMITH M, WARREN F, BYSTRYN J-C, FROHMAN L.** Skin biopsies in the evaluation of atypical optic neuropathies. In: Ocular Immunology and Inflammation, Aeolus Press Buren (The Netherlands), 1(3):231-241, 1993.
143. **BYSTRYN J-C, SHAPIRO R, ROSES DF, ORATZ R.** Melanoma vaccines. *Hem/Onc Annals*, 1(4):301-307, 1993.
144. **HANN SK, KIM HI, IM S, PARK YK, CUI J, BYSTRYN J-C.** The change of melanocyte cytotoxicity after systemic steroid treatment in vitiligo patients. *J Dermatol Sci* 6(3):201-5, 1993.

**BYSTRYN, JEAN-CLAUDE**  
Curriculum vitae

Page - 11 -

143. BAER RL, BYSTRYN J-C, COHEN D, CURWIN J, FREEDBERG IM, KOPF AW, LIPKIN G, PODWAL M, SHUPACK J. A tribute to Bernie. *Amer J Dermatopathol* 18(1):89-96, 1994.
144. GAO S-Q, BYSTRYN J-C. Identification of a novel basement membran antigen (p84) defined by sera with antibodies to both th epidermal and dermal side of split skin. *J of Invest Derm* 102:236-240, 1994.
147. JOHNSTON D, EL ROUBY S, BYSTRYN J-C. Identification of melanoma cell surface antigens Immun genic in mice. *Cancer Biotherapy* 9:29-36, 1994.
148. TOBIN DJ, ORENTREICH N, BYSTRYN J-C. Autoantibodies to hair follicles in normal individuals. *Arch Dermatol* 130:395-398, 1994.
149. TOBIN DJ, ORENTREICH N, FENTON DA, BYSTRYN J-C. Antibodies to hair follicles in alopecia areata. *J of Invest Derm* 102:721-724, 1994.
150. GERSHMAN N, JOHNSTON D, BYSTRYN J-C. Potentiation of B16 melanoma vaccine immunogenicity by IL-2 liposomes. *Vaccine Res* 3(2):83-92, 1994.
151. GAO S-Q, BYSTRYN J-C. A novel bullous pemphigoid antigen (BP125) located in the deeper layers of the basement membrane zone. *Arch Dermatol* 130(7):673-686, 1994.
152. HENN M, SHROBA S, BYSTRYN J-C. Comparison of immunogenic melanoma antigens defined by human and murine antibodies. *Vaccine Res* 3:107-116, 1994.
153. CUI J, CHEN D, ORATZ R, ZELENIUCH-JACQUOTTE A, HARRIS M, ROSES D, BYSTRYN J-C. Induction of cytolytic antibodies to melanoma by immunization to a polyvalent melanoma antigen vaccine. *Vaccine Res* 3(4):175-182, 1994.
154. CUI J, CHEN D, MISFELDT M, SWINFARD R, BYSTRYN J-C. Antimelanoma antibodies in swin with spontaneously regressing melanoma. *Pigment Cell Res* 8:60-63, 1995.
155. BYSTRYN J-C, SHAPIRO RL, ORATZ R. Cancer vaccines: Clinical applications: Partially purified tumor antigen vaccines. In: Biologic Therapy of Cancer, 2nd Edition, ed by V DeVita, S Hellman, and SA Rosenberg; JB Lippincott, Philadelphia, PA, pp 668-679, 1995.
156. TOBIN DJ, COLEN SR, BYSTRYN J-C. Isolation and long term culture of human hair-follicle melanocytes. *J Invest Dermatol* 104:86-89, 1995.
157. CUI J, BYSTRYN J-C. Melanoma and vitiligo are associated with antibody responses to similar antigens on pigment cells. *Arch Dermatol* 131:314-18, 1995.
158. CUI J, ARITA Y, BYSTRYN J-C. Characterization of vitiligo antigens. *Pigment Cell Res* 8:53-59, 1995.
159. BYSTRYN J-C. Clinical activity of a polyvalent melanoma antigen vaccine. In: Skin Cancer: Basic Sciences, ed by C Garbe; Springer-Verlag, Berlin, Heidelberg; *Recent Results in Cancer Research* 139:338-48, 1995.
160. MILLER K, ABELES G, ORATZ R, ZELENIUCH-JACQUOTTE A, CUI J, ROSES DF, HARRIS M, BYSTRYN J-C. Improved survival of melanoma patients with an antibody response to immunization to a polyvalent melanoma vaccine. *Cancer* 75(2):495-502, 1995.
161. BYSTRYN J-C. Advances in melanoma vaccine development. *The Skin Cancer Foundation Journal* Vol XIII:21, 90-91, 1995.
162. SCHULTZ N, ORATZ R, CHEN D, JACQUOTTE A, ABELES G, BYSTRYN J-C. Effect of DETOX as an adjuvant for melanoma vaccine. *Vaccine* 13(5):503-508, 1995.



**BYSTRYN, JEAN-CLAUDE**  
*cutaneous vice*

Page - 12 -

163. TOBIN DJ, BYSTRYN J-C. Immunity to hair follicles in alopecia areata. *J Invest Derm* 104(5):13S-14S, 1995.
164. ADLER A, ORATZ R, BYSTRYN J-C. In vitro cell-mediated immune responses induced by a polyvalent allogeneic melanoma vaccine. *Cancer Biother* 10(3):211-24, 1995.
165. NIIMI Y, IOANNIDES D, BUYON J, BYSTRYN J-C. Heterogeneity in the expression of Ro and La antigens in human skin. *Arthritis & Rheumatism* 38:1271-76, 1995.
166. BYSTRYN J-C, STEINMAN NM. The adjuvant therapy of pemphigus. An update. *Archives of Derm* 132:203-212, 1996.
167. BYSTRYN J-C, SHAPIRO RL, HARRIS M, ROSES DF, ORATZ R. The use of vaccines in the treatment of malignant melanoma. *Clinics in Dermatology* 14:337-41, 1996.
168. TOBIN DJ, BYSTRYN J-C. Alopecia areata is associated with antibodies to hair follicle-specific antigens located predominantly in the proliferative region of hair follicles. In: Hair Research for the Next Millenium, ed by D Van NESTE and VA Randall; Elsevier, Amsterdam, Netherlands, pp 237-41, 1996.
169. BYSTRYN J-C, TOBIN DJ, HANN S-K, SONG M-S. Hair follicle structures targeted by antibodies in alopecia areata. In: Hair Research for the Next Millenium, ed by D Van NESTE and VA Randall; Elsevier, Amsterdam, Netherlands, pp 233-36, 1996.
170. BYSTRYN J-C. Erythema multiforme with mucous membrane involvement and Stevens-Johnson Syndrome are clinically different disorders. *Arch Dermatol* 132:711, 1996.
171. ORATZ R, BYSTRYN J-C. Malignant Melanoma. In: Immunomodulatory and Cytotoxic Agents in Dermatology, ed by CJ McDonald; Marcel Dekker, New York, NY, In press.
172. TOBIN DJ, HANN S-K, SONG M-S, BYSTRYN J-C. Hair follicle structures targeted by antibodies in alopecia areata. In press, *Archives of Derm*.

#### **SUBMITTED FOR PUBLICATION**

173. HANN S-K, REYNOLDS SR, BYSTRYN J-C. Detection of antibodies to melanocytes in vitiligo by Western immunoblotting.
174. APPLEBAUM J, REYNOLDS S, KNISPEL J, ORATZ R, SHAPIRO RL, BYSTRYN J-C. Induction of antibodies to tumor antigens expressed in vivo by immunization to a shed polyvalent melanoma vaccine.
175. CUNHA PR, JIAO D, BYSTRYN J-C. Simultaneous occurrence of herpetiform pemphigus and endemic pemphigus foliaceus (fogo selvagem).
176. TOBIN DJ, SUNDBERG JP, KING LE JR, BOGGESS D, BYSTRYN J-C. Autoantibodies to hair follicles in C3H/HeJ mice with alopecia areata-like hair loss.
177. JIAO D, BYSTRYN J-C. Sensitivity of indirect immunofluorescence, substrate specificity and immunoblotting in the diagnosis of pemphigus.
178. HERBST A, BYSTRYN J-C. Remissions in pemphigus.
179. JIAO D, BYSTRYN J-C. Antibodies to desmoplakin in a patient with non-neoplastic pemphigus foliaceus.
180. ABDEL-NASER MB, HANN S-K, BYSTRYN J-C. PUVA therapy releases circulating growth factor(s) that stimulate cell proliferation.
181. REYNOLDS SR, ORATZ R, SHAPIRO RL, FOTINO M, HAO P, VUKMANOVIC S, BYSTRYN J-C. A melanoma vaccine induces MAGE-3 and MART-1/MELAN-A specific CD8+ T cells that correlate with clinical outcome.

**BYSTRYN, JEAN-CLAUDE**  
curriculum vitae

page - 13 -

## ABSTRACTS

1. **BYSTRYN J-C.** Antibodies to human melanoma demonstrated by a new radioimmunoassay. *J Invest Derm* 58:250, 1972.
2. **BYSTRYN J-C, BART RS, LIVINGSTON P, KOPF AW.** Immunity to murine melanoma. *Clin Res* 21:476, 1973.
3. **BYSTRYN J-C, ABEL E, DEFEO C.** Epidermal antibodies of unique specificity in pemphigus foliaceus. *J Invest Derm* 62:543, 1974.
4. **BYSTRYN J-C, SCHENKEIN I, UHR, JW.** Radioimmunoassay of antibodies to, and partial isolation and characterization of, antigens associated with murine melanoma. *Clin Res* 22:485A, 1974.
5. **RODRIGUEZ J, BYSTRYN J-C.** Reliability of the indirect immunofluorescent test for pemphigus and bullous pemphigoid. *Clin Res* 23:454A, 1975.
6. **BYSTRYN J-C, SCHENKEIN I.** Partial isolation and characterization of antigens associated with murine melanoma. IXth Int'l Pigment Cell Conference, Houston, TX, pp 9, 1975.
7. **BYSTRYN J-C, SCHENKEIN I.** Specificity and immunogenicity of antigens associated with murine melanoma. *Proc Am Assoc Cancer Res* 16:379, 1975.
8. **BYSTRYN J-C.** Antigens associated with murine melanoma. *J Invest Derm* 64:266, 1975.
9. **BYSTRYN J-C.** Release of tumor associated antigens by murine melanoma cells. *Proc Am Assoc Cancer Res* 17:216, 1976.
10. **ABEL E, BYSTRYN J-C.** Epidermal cytoplasmic antibodies: incidence and type in normal persons and in patients with melanoma. *Clin Res* 24:94A, 1976.
11. **BYSTRYN J-C.** Clinical significance of basal cell antibodies. *Clin Res* 24:262A, 1976.
12. **BYSTRYN J-C, FRANCES C.** Cytoplasmic differentiation antigens of human epidermal cells. *Proc Fed Am Soc for Exp Biol* 36:1312, 1977.
13. **BYSTRYN J-C.** Identification of soluble cell-surface antigens by human antigens. *Clin Res* 25:280A, 1977.
14. **BYSTRYN J-C.** Release of cell-surface antigens by human melanoma cells. *Proc Am Assoc Cancer Res* 18:7, 1977.
15. **BYSTRYN J-C, RODRIGUEZ J.** Pemphigus foliaceus associated with absence of intercellular antigens in the deep layers of the epidermis. *Clin Res* 25:280A, 1977.
16. **BYSTRYN J-C, FRANCES C.** Differentiation antigens of human epidermal cells. *Clin Res* 26:374A, 1978.
17. **SMALLEY JR, BYSTRYN J-C.** Purification of cell-surface glycoprotein from human melanoma. *Fed Proc* 37:1595, 1978.
18. **SMALLEY JR, BYSTRYN J-C.** Isolation of a human melanoma associated cell-surface antigen. *Proc XII Int'l Cancer Congress, Buenos Aires*, 1978.
19. **BYSTRYN J-C, SMALLEY JR, PERLSTEIN J.** Autocatabolism of cell-surface macromolecules and melanoma associated antigens (MAA) released by human melanoma cells. *Proc XII Int'l Cancer Congress, Buenos Aires*, 1978.
20. **BYSTRYN J-C, SMALLEY JR, PERLSTEIN J.** Degradation of shed cell-surface macromolecules and melanoma associated antigens (MAA) by human melanoma cells. *Proc Am Assoc Cancer Res* 19:128, 1978.

**BYSTRYN, JEAN-CLAUDE**  
*curriculum vitae*

page - 14 -

21. **BYSTRYN J-C, SMALLEY JR.** Purification of cell-surface human melanoma associated antigens. Clin Res 26:432A, 1978.
22. **BYSTRYN J-C, PERLSTEIN J.** Autocatabolism of cell-surface macromolecules shed by melanoma cells. J Invest Derm 72:266, 1979.
23. **BHAVANADAN VP, KEMPER J, BYSTRYN J-C.** Purification and partial characterization of a B16 mouse melanoma antigen. The XI Int'l Congress of Biochem, Toronto, Canada, pp 521, 1979.
24. **BOCTOR AM, BYSTRYN J-C.** Degradation of cell-surface melanoma associated antigens (MAA) shed by human melanoma cells. Proc Am Assoc Cancer Res 21:250, 1980.
25. **JACOBS MI, SCHNED ES, BYSTRYN J-C.** Variability of the lupus band test (LBT) in adjacent skin sites of SLE patients. Presented at Vllth Northeastern Regional Meeting of the American Rheumatism Assoc, 1980.
26. **BOCTOR AM, BYSTRYN J-C.** Degradation of soluble melanoma associated antigens. Yale J of Biol Med 53:477-478, 1980.
27. **BYSTRYN J-C, HOWANITZ N, NORDLUND J, LERNER AB.** Antibodies to melanocytes in patients with vitiligo. Yale J Biol Med 53:477-478, 1980.
28. **BYSTRYN J-C, TEDHOLM CA.** Shedding of human melanoma associated antigens (MAA) with plasma membrane fragments. Fed Proc 40:981, 1981.
29. **HEANEY-KIERAS J, BYSTRYN J-C.** Purification of a 75K d cell-surface human melanoma associated antigen (MAA). Proc 17th Annual Meeting Am Soc Clin Onc 22:286, 1981.
30. **ZHU X-J, EISINGER M, BYSTRYN J-C.** Characterization of surface macromolecules on human keratinocytes. J Invest Derm 76:316, 1981.
31. **HOWANITZ N, NORDLUND J, LERNER A, BYSTRYN J-C.** Antibodies to melanocytes in patients with vitiligo. J Invest Derm 76:323, 1981.
32. **HEANEY-KIERAS J, BYSTRYN J-C.** Purification of a M = 75,000 cell-surface human melanoma associated antigen (MAA). Presented at 1981 annual meeting of the Soc. Complex Carbohydrates, Philadelphia, Pennsylvania, 1981.
33. **HEANEY-KIERAS J, BYSTRYN J-C.** Identification and purification of a 75 kD tumor antigen shed from the surface of human melanoma cells. Clin Res 30:262A, 1982.
34. **ZHU X-J, BYSTRYN J-C.** Heterogenicity of bullous pemphigoid antigens. Clin Res 30:616A, 1982.
35. **JOHNSTON D, BYSTRYN J-C.** Mechanism of autodegradation of surface macromolecules shed by human melanoma cells. Fed Proc 41(3):733, 1982.
36. **LAMBERG SI, GREEN SB, BLOCK JB, BYSTRYN J-C, et al.** Definitive clinical staging of cutaneous T-cell lymphomas. Clin Res 30:263A, 1982.
37. **BYSTRYN J-C.** Specific immunotherapy of melanoma. NYU Cancer Report 6:8-9, 1982.
38. **ZIMMERMAN J, JOHNSTON D, BYSTRYN J-C.** A glycolipid antigen selectively expressed by human melanoma cells. J Cell Biol 95:267A, 1982.
39. **JOHNSTON D, BYSTRYN J-C.** Effect of tunicamycin on release of macromolecules and melanoma-associated antigens (MAAs). Fed Proc 42:2129, 1983.

**BYSTRYN, JEAN-CLAUDE**  
CURRICULUM VITAE

page - 15 -

40. HEANEY-KIERAS J, BYSTRYN J-C. Purification of a 112 KD and 128 KD cell-surface human melanoma associated antigen (MAAs). Proc Am Assoc Cancer Res 24:233, 1983.
41. LIU P, VALENTINE F, BYSTRYN J-C. Surface properties of melanoma cells in different metastases in the same individual. Clin Res 31:265A, 1983.
42. GOLDBERG DJ, BYSTRYN J-C. Regional variation in the expression of bullous pemphigoid antigen in human skin. Clin Res 31:568A, 1983.
43. NAUGHTON GK, EISINGER M, BYSTRYN J-C. Antibodies to melanocytes in vitiligo. Clin Res 31:951A, 1983.
44. BYSTRYN J-C. Development of a polyvalent melanoma tumor-antigen vaccine. J Derm Surg 9:648-649, 1983.
45. BYSTRYN J-C, NAUGHTON GK. Immunogenic human melanoma antigens defined by antibodies in patients with vitiligo. Proc Am Assoc Cancer Res 25:272, 1984.
46. BYSTRYN J-C, LEVIN M, BERNSTEIN P, SPEYER J, WOLSK D. Immunogenicity of a polyvalent melanoma tumor antigen vaccine in man. Proc Am Assoc Cancer Res 25:229, 1984.
47. BYSTRYN J-C, LEVIN M, SPEYER S, HARRIS M, ROSES D, BERNSTEIN P. Phase I trial of specific immunotherapy of melanoma with a polyvalent melanoma antigen vaccine. Clin Res 32:574A, 1984.
48. NAUGHTON GK, EISINGER M, BYSTRYN J-C. Identification and partial characterization of vitiligo antigens. Clin Res 32:605A, 1984.
49. BYSTRYN J-C, BERNSTEIN P, VALENTINE F. Strategies to circumvent antigenic heterogeneity restricting the specific immunotherapy of melanoma. Clin Res 32:481A, 1984.
50. BYSTRYN J-C, NAUGHTON GK. Immunity to pigmented cells in vitiligo and melanoma. Fed Proc 43:1683, 1984.
51. JOHNSTON D, BYSTRYN J-C. Human melanoma-associated glycolipid antigens immunogenic in man. Proc Am Assoc Cancer Res. 25:1100, 1984.
52. REGGIARDO D, NAUGHTON GK, BYSTRYN J-C. Relation to titer of pigment cell antibodies to extent of depigmentation in patients with vitiligo. Clin Res 32:811A, 1984.
53. NAUGHTON GK, MAHAFFEY M, BYSTRYN J-C. Antibodies to surface antigens of pigmented cells in animals with vitiligo. Clin Res 32:257A, 1984.
54. LIPKIN G, NAUGHTON GK, ROSENBERG M, BYSTRYN J-C. Expression of vitiligo-related pigment cell differentiation antigens on melanoma cells after phenotypic reversion by contact inhibitory factor. Clin Res 32:713A, 1985.
55. LIPKIN G, NAUGHTON GK, ROSENBERG M, BYSTRYN J-C. Expression of vitiligo-related pigment cell reversion by contact inhibitory factor. Clin Res 33:662A, 1985.
56. BYSTRYN J-C, BERNSTEIN P, HARRIS M, ROSES D, SPEYER J. Effects of a polyvalent tumor antigen vaccine in human malignant melanoma. Clin Res 33:626A, 1985.
57. GOLDBERG DJ, TORRES D, BYSTRYN J-C. Antibodies to epidermal cytoplasmic antigens following treatment of multiple basal cell carcinomas. Clin Res 33:640A, 1985.
58. NAUGHTON GK, BYSTRYN J-C. Immunological relationship between vitiligo and melanoma. Clin Res 33:669A, 1985.

**BYSTRYN, JEAN-CLAUDE**  
curriculum vitae

page - 18 -

59. SISON FONCIER L, SABOLINSKI M, BYSTRYN J-C. Regional variation in the expression of pemphigus vulgaris antigen in human skin. Clin Res 33:663A, 1985.
60. LIPKIN G, ROSENBERG M, NAUGHTON G, BYSTRYN J-C, NABI ZF, ZUCKER-FRANKLIN D. Phenotypic reversion of melanoma by contact inhibitory factor - experimental approaches to growth control. (Abstracts) Third European Conference on Clinical Oncology and Cancer Nursing, Stockholm, p 72, June 20, 1985.
61. NAUGHTON GK, BYSTRYN J-C. Immunological relationship between vitiligo and melanoma. J Invest Derm 84:289, 1985.
62. LIPKIN G, NAUGHTON GK, ROSENBERG M, BYSTRYN J-C. Vitiligo-related pigment cell differentiation antigens are expressed on malignant melanoma cells following phenotypic reversion induced by contact inhibitory factor. Differentiation 30:35-39, 1985.
63. BYSTRYN J-C, BERNSTEIN P, HARRIS M, ROSES D, SPEYER J. Effects of a polyvalent tumor antigen vaccine in human malignant melanoma. J Invest Derm 84:338, 1985.
64. GOLDBERG DJ, TORRES D, BYSTRYN J-C. Antibodies to epidermal cytoplasmic antigens following treatment of multiple basal cell carcinomas. J Invest Derm 84:382, 1985.
65. BYSTRYN J-C, LONBERG M, BERNSTEIN P, HARRIS M, ROSES D, SPEYER J. Immunogenicity of a polyvalent melanoma antigen vaccine in patients with early melanoma. Proc Am Assoc Cancer Res 26:1233, 1985.
66. JOHNSTON D, SCHACHNE JP, BYSTRYN J-C. Identification of B16 melanoma-associated antigens immunogenic in syngeneic mice. Fed Proc Am Soc Exp Biol 44:797, 1985.
67. BYSTRYN J-C. Introduction: Workshop on immunology, chemotherapy and photobiology. J Derm Surg Oncol 11:775, 1985.
68. BYSTRYN J-C. Treatment of melanoma with a polyvalent melanoma antigen vaccine. J Dermatol Surg Oncol 1:778, 1985.
69. BYSTRYN J-C, ORTAZ R, HARRIS MN, ROSES DF, SPEYER J. Cellular immune response to a melanoma antigen vaccine. Clin Res 43, 2:561A, 1985.
70. BONFA E, BYSTRYN J-C, ELKON EB. Comparison between autoantibodies in autoimmune, infectious and neoplastic disease. Arthritis and Rheumatism 29:54, 1986.
71. JOHNSTON D, BYSTRYN J-C. Development of a melanoma vaccine: Comparative immunogenic and tumor protective activity of B16 melanoma vaccines. Proc Am Assoc Can Res. 27:1414, 1986.
72. NORRIS DA, KISSINGER RM, BYSTRYN J-C, NAUGHTON G. Vitiligo patients' sera induce complement-mediated lysis and ADCC of human melanocytes in vitro. Clin Res 34, 2:772A, 1986.
73. SELTER JH, BYSTRYN J-C. Correlation between antibody titer and disease activity in bullous pemphigoid. Clin Res 34, 2:780A, 1986.
74. SISON-FONACIER LM, BYSTRYN J-C. Heterogeneity of pemphigus vulgaris antigens. Clin Res 34, 2:781A, 1986.
75. HESSLE H, BURNETT KG, BYSTRYN J-C. Tumor vaccine induced human monoclonal antibodies to melanoma. In: Proc 6th International Congress of Immunology, Toronto, 1986.
76. NORRIS DA, BYSTRYN J-C, KISSINGER RM. Direct evidence for immunologic cytotoxicity as a mechanism of human vitiligo. Clin Res 35:2-51A, 1987.

**BYSTRYN, JEAN-CLAUDE**  
curriculum vitae

page - 17 -

77. ORATZ R, COCKERELL C, SPEYER J, ROSES DF, HARRIS MN, BYSTRYN J-C. Induction of lymphocytic cell infiltrate in human melanoma nodules by active immunization to melanoma antigen vaccine. Proc Am Assoc Can Res 28:574, 1987.
78. DUGAN M, ORATZ R, SPEYER J, ROSES DF, HARRIS MN, GOLOMB F, BYSTRYN J-C. Relation between immune response to polyvalent melanoma vaccine and tumor progression in patients with stage II melanoma. Clin Res 35:523, 1987.
79. BYSTRYN J-C. Melanoma Antigen Vaccine. Dermatology in Five Continents, Proceedings of the XVII World Congress of Dermatology, Berlin, May 24-29, pp 691-692, 1987.
80. DUGAN M, ORATZ R, HARRIS MN, ROSES DF, SPEYER J, GOLOMB F, HENN M, BYSTRYN J-C. Delayed tumor progression in melanoma patients with cellular immune response to melanoma vaccine. Clin Res 36:495A, 1988.
81. BYSTRYN J-C, DUGAN M, ORATZ R, SPEYER J, ROSES DF, HARRIS MN. Potentiation of human melanoma vaccine: Relation between method of immunization, immunogenicity, and tumor progression. J of Cellular Biochemistry 12E:202T, 1988.
82. CHIN J, HENN M, BYSTRYN J-C. Protein antigens defined by antibodies in patients with melanoma. Fed of Amer Soc Exp Biol 2:1838A, 1988.
83. JOHNSTON D, ULRICH TJ, SOUDREAU CE, BYSTRYN J-C. Detox<sup>TM</sup> enhances humoral response to B16 melanoma antigen vaccine. Fed of Amer Soc Exp Biol 2:1841A, 1988.
84. NIIMI Y, TANNER S, BYSTRYN J-C. Expression of Ro and La antigens in sun exposed and sun protected human skin. Clin Res 37:2:781A, 1989.
85. ZHU XJ, NIIMI Y, BYSTRYN J-C. Identification of bullous pemphigoid antigens by immunoblotting technique. Clin Res 37:2:771A, 1989.
86. ORATZ R, DUGAN M, HARRIS MN, ROSES DF, SPEYER J, HOCHESTER H, HENN M, BLUM RH, BYSTRYN J-C. A randomized trial of an adjuvant polyvalent melanoma antigen vaccine with or without cyclophosphamide in patients with malignant melanoma. Proc of the Amer Soc of Clin Oncol 8:288, 1989.
87. BYSTRYN J-C, ORATZ R, HARRIS MN, ROSES DF, SPEYER J. Potentiation of immune response to a melanoma antigen vaccine. In: Vaccines: New Concepts and Developments, ed. by H Kohler and PT LoVerde; John Wiley and Sons, New York, pp 438-439, 1989.
88. IOANNIDES D, HYTIROGLOU P, PHELPS R, BYSTRYN J-C. Differences in regional expression of pemphigus foliaceus, erythematosus and vulgaris antigens in human skin. Clin Res 38:626A, 1990.
89. ZHU X-J, NIIMI Y, BYSTRYN J-C. Identification of a 160 kD molecule as a component of the basement membrane zone and as a minor bullous pemphigoid antigen. Clin Res 38:607A, 1990.
90. CUI J, HARNING R, LI J, BYSTRYN J-C. Analysis of pigment cell antigens defined by vitiligo antibodies. Clin Res 38:623A, 1990.
91. NIIMI Y, ZHU X-J, BYSTRYN J-C. Identification of antigens defined by BMZ antibodies that react to both the epidermal and dermal side of 1M NaCl split skin. Clin Res 38:605A, 1990.
92. LI J, HENN M, ORATZ R, BYSTRYN J-C. The antibody response to immunization to a polyvalent melanoma antigen vaccine. Clin Res 38:660A, 1990.
93. ADLER A, ORATZ R, LIEBES L, BYSTRYN J-C. Effect of allogeneic melanoma vaccine on the in vitro cellular immune response. Proc Am Assoc Cancer Res 31:252, 1990.

**BYSTRYN, JEAN-CLAUDE**  
curriculum vitae

page - 18 -

94. EL ROUBY S, BYSTRYN J-C. In vitro analysis of the immunogenicity of B16 melanoma vaccine. Proc Am Assoc Cancer Res 31:254, 1990.
95. BYSTRYN J-C, U J, HENN M, ORATZ R. Identification of immunogenic antigens in a polyvalent melanoma antigen vaccine. Proc Am Assoc Cancer Res 31:280, 1990.
96. BYSTRYN J-C, ORATZ R, HARRIS MN, ROSES DF, HENN M. The DTH response to melanoma vaccine immunization. J of Cellular Biochemistry. Supp 14B:105, 1990.
97. BYSTRYN J-C, ORATZ R, ROSES DF, HARRIS MN, HENN M, LEW R. Improved survival of melanoma patients with delayed type hypersensitivity response to melanoma vaccine immunization. Clin Res 39:503A, 1991.
98. IOANNIDES D, BYSTRYN J-C. Pemphigus antibodies react to 56, 67 and 85 kDs epidermal antigens. Clin Res 39:479A, 1991.
99. CUI J, ORATZ R, HARRIS M, ROSES D, HENN M, BYSTRYN J-C. Induction of cytolytic melanoma antibodies by immunization to a polyvalent melanoma antigen vaccine. Proc Am Assoc Cancer Res 32:256, 1991.
100. CUI J, ALITA Y, EISINGER M, BYSTRYN J-C. Killing of melanocytes by vitiligo antibodies. Clin Res 39:552A, 1991.
101. JOHNSTON D, BYSTRYN J-C. Enhancement of immunity to B16 melanoma vaccine by the adjuvant SAF-M. Proc Am Assoc Cancer Res 32:25, 1991.
102. ORATZ R, ROSES D, HARRIS M, ZEIRE E, BYSTRYN J-C. Effect of DETOX on the immunogenicity of a melanoma vaccine. Proc of the American Society of Clinical Oncology, 10:296, 1991.
103. FROHMAN L, BIELORY L, WARREN F, BYSTRYN J-C, KUPERSMITH M. Skin biopsies in the evaluation of atypical optic neuropathies. Assoc. of Research in Vision and Ophthalmology (ARVO), Annual Meeting on Investigative Ophthalmology and Visual Science, 32:951, 1991.
104. SCHULTZ N, ORATZ R, CHEN D-L, BYSTRYN J-C. Effect of DETOX on the antibody response to a polyvalent melanoma antigen vaccine in man. Clin Res 39:675A, 1991.
105. BYSTRYN J-C, CUI J, ARITA Y, EISINGER M. Melanoma and vitiligo are associated with antibody responses to similar pigment cell antigens. Clin Res 40(2):507A, 1992.
106. CUI J, ARITA Y, EISINGER M, BYSTRYN J-C. Characterization of vitiligo antigens. Clin Res 40(2):455A, 1992.
107. JOHNSTON D, ORLOW SJ, BYSTRYN J-C. Upregulation of immunogenic murine melanoma antigen expression. Proc Am Assoc Cancer Res 33:321, 1992.
108. GERSHMAN N, JOHNSTON D, BYSTRYN J-C. Enhancement of immunity to B16 melanoma by IL-2 liposomes. Clin Res 40:684A, 1992.
109. CUI J, CHEN D, MISFELDT M, SWINFARD RW, BYSTRYN J-C. Antimelanoma antibodies in swines with spontaneously regressing melanoma. Proc Am Assoc Cancer Res 33:351, 1992.
110. SCHULTZ N, ORATZ R, CHEN D-L, BYSTRYN J-C. Effect of DETOX on the immunogenicity of a polyvalent melanoma antigen vaccine in man. Proc of the American Society of Clinical Oncology 11:1160, 1992.
111. GAO S-Q, BYSTRYN J-C. Identification of a dermal antigen (p84) defined by combined staining sera. Clin Res 41(2):409A, 1993.
112. CUI J, CHEN D-L, MISFELDT M, SWINFARD RW, BYSTRYN J-C. Pigment cell antimelanoma antibodies in swine with melanoma and vitiligo. Clin Res 41(2):408A, 1993.

**BYSTRYN, JEAN-CLAUDE**  
CURRICULUM VITAE

page - 10 -

113. TOBIN DJ, BYSTRYN J-C. Autoimmunity to hair follicles in normal individuals. Clin Res 41(2):472A, 1993.
114. BYSTRYN J-C, MILLER K, CUI J, ORATZ R. Identification of candidate antigens for construction of melanoma vaccines. Clin Res 41(2):489A, 1993.
115. MILLER K, ABELES G, ORATZ R, CUI J, ROSES DF, HARRIS MN, BYSTRYN J-C. Prolongation of survival of melanoma patients with antibody response to immunization with a polyvalent melanoma vaccine. Proc Am Soc Clin Res 12:1353, 1993.
116. TOBIN DJ, BYSTRYN J-C. Normal individuals have antibodies to hair follicle antigens which are modulated during the hair growth cycle. J Invest Dermatol 101(3):451, 1993.
117. BYSTRYN J-C, ORATZ R, HARRIS MN, ROSES DF. Clinical activity of a polyvalent melanoma vaccine. J Invest Dermatol 101(3):396, 1993.
118. GAO S-Q, BYSTRYN J-C. A novel bullous pemphigoid antigen (BP125) is located in the deeper layers of the basement membrane zone. J Invest Dermatol 101(3):481, 1993.
119. BYSTRYN J-C, MILLER K, ABELES G, ORATZ R, ROSES DF, HARRIS MN. Improved survival of melanoma patients with antibody responses to a polyvalent melanoma vaccine. Melanoma Res 3:51, 1993.
120. ADLER A, ORATZ R, BYSTRYN J-C. Characterization of in vitro cellular immune responses induced by a polyvalent melanoma antigen vaccine. Specific Immunotherapy of Cancer with Vaccines. Washington, DC, 1993.
121. MILLER K, ABELES G, ORATZ R, BYSTRYN J-C. Characterization of candidate human melanoma antigens for vaccine construction. Specific Immunotherapy of Cancer with Vaccines. Washington, DC, 1993.
122. BYSTRYN J-C, ORATZ R, HARRIS MN, ROSES DF. Clinically beneficial immune responses to melanoma vaccine treatment. Third International Conference on Melanoma. Venice, 1993.
123. JOHNSTON D, GERSHMAN N, BYSTRYN J-C. IL-2 liposomes enhance responses to a B16 melanoma vaccine. First International Conference on Engineered Vaccines. San Francisco, 1993.
124. BYSTRYN J-C, MILLER K, ABELES G, ORATZ R, ZELENIUCH-JACQUOTTE A, ROSES DF, HARRIS M. Mechanism of action of melanoma vaccines. Proc of the American Society of Clinical Oncology 13:976, 1994.
125. ORATZ R, SHAPIRO RL, CHEN D, HARRIS M, ROSES D, BYSTRYN J-C. Effect of IL-2 liposomes on the immunogenicity of a polyvalent melanoma antigen vaccine. Proc of the American Society of Clinical Oncology 13:1350, 1994.
126. BYSTRYN J-C, TOBIN DJ. Alopecia areata is associated with antibodies to hair follicle melanocytes. J Invest Dermatol 102(4):532, 1994.
127. TOBIN DJ, ORENTREICH N, FENTON DA, BYSTRYN J-C. Antibodies to hair follicles in alopecia areata. J Invest Dermatol 102(4):591, 1994.
128. ABDEL-NASER MB, HANN SK, BYSTRYN J-C. PUVA therapy releases circulating growth factor(s) that stimulate melanocyte proliferation. J Invest Dermatol 102(4):649, 1994.
129. JOHNSTON D, RIVAS M, BYSTRYN J-C. QS-21 enhances tumor protection and immune response to B16 melanoma vaccines. Proc Am Assoc Cancer Res 35:489, 1994.
130. BYSTRYN J-C. Characterization of "vitiligo" antigens. PASPCR 3:11 (7), 1994.
131. TOBIN DJ, COLEN SR, BYSTRYN J-C. Isolation and long term culture of human-hair follicle melanocytes. Pigment Cell Res 3:23 (54), 1994.



**BYSTRYN, JEAN-CLAUDE**  
curriculum vitae

page - 20 -

132. TOBIN DJ, BYSTRYN J-C. Natural autoantibodies to cycle-specific hair follicle antigens. *J Invest Dermatol* 101(3):451, 1994.
133. BYSTRYN J-C, ORATZ R, SHAPIRO RL, ROSES D, HARRIS M, JOHNSTON D. Vaccine treatment of melanoma. 38th Annual Clinical Conference of the Advances in the Biology and Clinical Management of Melanoma. Houston, University of Texas MD Anderson Cancer Center, pp 53-54, 1995.
134. SHAPIRO RL, ORATZ R, ROSES DF, HARRIS MN, BYSTRYN J-C. Surgical resection of distant melanoma metastases prior to immunotherapy with a polyvalent melanoma antigen vaccine encapsulated into liposomes with IL-2. *Proc Am Soc Oncology*, 1995. - GET EXACT REF
135. HANN S-K, CHEN D, BYSTRYN J-C. Systemic steroids suppress melanocyte antibodies in vitiligo. *J Invest Dermatol* 104(4):564, 1995.
136. TOBIN DJ, BYSTRYN J-C. Identification and tissue localization of hair follicle antigens targeted by antibodies in alopecia areata. *J Invest Dermatol* 104(4):578, 1995.
137. BYSTRYN J-C. Immune responses to melanoma. *Dermatol Surg* 21:900-906, 1995.
138. TOBIN DJ, BYSTRYN J-C. Alopecia areata is associated with antibodies to 44/46 kD hair-specific keratins located in the hair bulb and lower follicle. First Tricontinental Meeting of Hair Research Societies, 1995.
139. BYSTRYN J-C, TOBIN DJ. Hair follicle structures targeted by antibodies in alopecia areata. First Tricontinental Meeting of Hair Research Societies, 1995.
140. BYSTRYN J-C. Immunopathogenesis of alopecia areata. Proceedings of the 9th Korea-Japan Joint Meeting of Dermatology 109-112, 1995.
141. HERBST A, BYSTRYN J-C. Remissions in pemphigus. *J Invest Derm* 106(4):850 (270), 1996.
142. TOBIN DJ, SUNDBERG JP, KING LE, BOGGESS D, BYSTRYN J-C. Autoantibodies to hair follicles in the C3H/HeJ mouse model of alopecia areata. *J Invest Derm* 106(4):838 (802), 1996.
143. XIE Z, BYSTRYN J-C. Anti-tyrosinase antibodies in vitiligo. *J Invest Derm* 106(4):838 (794), 1996.
144. BYSTRYN J-C, ORATZ R, SHAPIRO R, JOHNSTON D, HARRIS M, ROSES D, ZELENIUCH-JACQUOTTE A, CHEN DL, LAX A. Potentiation of melanoma vaccine activity by IL-2 liposomes. *J Invest Derm* 106(4):845 (235), 1996.
145. HANN S-K, TOBIN DJ, SONG M-S, BYSTRYN J-C. Hair follicle structures targeted by antibodies in alopecia areata. *J Invest Derm* 106(4):838 (795), 1996.
146. ROWILSON-CUNHA P, JIAO D, BYSTRYN J-C. Heterogeneity in intercellular antibodies in fogo selvagem. *J Invest Derm* 106(4):813 (45), 1996.
147. ORATZ R, SHAPIRO R, JOHNSTON D, HARRIS M, ROSES D, ZELENIUCH-JACQUOTTE A, CHEN D, LAX A, BYSTRYN J-C. IL-2 liposomes markedly enhance the activity of a polyvalent melanoma vaccine. *Proc Am Soc Clin Oncology* 15:438, 1996.
148. REYNOLDS SR, VUKMANOVIC S, ORATZ R, SHAPIRO RL, BYSTRYN J-C. A polyvalent melanoma vaccine induces a CD8 T cell response to MART-1 and MAGE-3 peptides. *Proc Am Assoc Cancer Res* 37:491, 1996.
149. BYSTRYN J-C. Effect of plasmapheresis therapy on circulating levels of pemphigus antibodies. International Conference for Apheresis, Kyoto 1996.
150. BYSTRYN J-C. Immunotherapy of melanoma with vaccines. Eurocancer 1996, Paris.

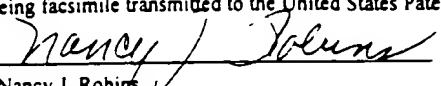
**BYSTRYN, JEAN-CLAUDE**  
curriculum vitae

page - 21 -

151. BYSTRYN J-C, ORATZ R, SHAPIRO R, REYNOLDS S, JOHNSTON D, HARRIS M, ROSES D, ZELENIUCH-JACQUOTTE A. Polyvalent melanoma antigen vaccine. Cambridg Symposia "Immunology and Immunotherapy of Metastasis," Lake Tahoe 1996.
152. BYSTRYN J-C, ORATZ R, SHAPIRO R, JOHNSTON D, REYNOLDS S, HARRIS M, ROSES D, ZELENIUCH-JACQUOTTE A. Potentiation of melanoma vaccin immunogenicity by IL-2 liposomes. Cambridg Symposia "Discovery and Development of Tumor Vaccines," Taos 1996.
153. BYSTRYN J-C. Clinical results with a SPAN melanoma antigen vaccine. Schering National Melanoma Faculty Meeting in "Current Issues in the Treatment of Melanoma," San Diego 1996.

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on November 4, 1996

  
Nancy J. Robius

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Lynn E. Spitler et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

**DECLARATION OF MICHAEL MASTRANGELO, MD  
PURSUANT TO 37 C.F.R § 1.132**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, Michael Mastrangelo, MD, declare as follows:

1. I am a Professor of Medicine, and Associate Clinical Director of the Jefferson Cancer Center in Philadelphia, Pennsylvania. A copy of my *Curriculum Vitae* is attached hereto as Exhibit A. I am Chairman of the Scientific Advisory Board of Jenner Technologies, the assignee in this application, and a shareholder in the company.

2. I have reviewed the Declaration Under 37 C.F.R. 1.132 prepared by Dr. Lynn E. Spitler describing the results of a clinical study directed to the use of prostate specific antigen (PSA) as an active ingredient in an antiprostata cancer vaccine. I am also familiar with the study itself, and with the results that were obtained.

3. The purpose of the study was to obtain evidence that the vaccines would raise a sufficient cellular immune response to have a beneficial effect with respect to prostate tumors. Such a result could be shown directly by measuring cytotoxic lymphocyte (CTL) generation; however, I am aware that this was not possible in these studies because the assay was not satisfactory because of the lack of an appropriate target cell for the assay.

4. The responses measured are understood in the art to be satisfactory substitutes for measuring CTLs. Thus, the proliferation of lymphocytes from two of the patients in response to contact with PSA or in response to peptides representing putative PSA epitopes is indicative of an appropriate cellular immune response. The ability of PSA or PSA derived peptides to stimulate cytokine production -- i.e., gamma interferon and IL-4 production -- from lymphocytes in these patients also indicates that the cellular response is obtained specifically with respect to PSA. The observation of the development of a positive skin test response to PSA in one patient is also consistent with these observations showing the development of cell-mediated immunity in the patients.

5. In my opinion, the results obtained in this clinical study provide evidence that the vaccines are likely to be effective in exerting a beneficial effect on patients with prostate tumors or at risk for prostate tumors.

6. The efficacy shown for the vaccine tested in the foregoing clinical studies further provides evidence that analogous vaccines based on host tissue antigen, such as prostate specific membrane antigen (PSMA) and prostate acid phosphatase (PAP) would behave in a similar manner. It is also well known that if the entire antigen is effective as a vaccine, portions of the antigen will be effective as well, especially if manipulated by art-known methods to enhance their immunogenicity, such as by coupling them to carrier.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

October 10, 1996

  
\_\_\_\_\_  
Michael Mastrangelo, MD

Revised 12/93

# CURRICULUM VITAE

NAME: Michael J. Mastrangelo, M.D.

SOCIAL SECURITY NO: 210-28-9391

DATE OF BIRTH: October 3, 1938

MARITAL STATUS: Married 1964 - Ann Sundav

Children: David M. 1965  
Mark S. 1967  
Audrey A. 1970

## EDUCATION

Villanova University, Villanova, PA 1956-1960  
B.S. (Biology), Summa Cum Laude (1/696)  
The Johns Hopkins University School of Medicine, M.D. 1960-1964

## POSTGRADUATE TRAINING

Thomas Jefferson University Hospital, Philadelphia, PA  
Medical Intern 1964-1965  
Medical Resident 1967-1969  
American Cancer Society Trainee 1968-1969  
Oncology Fellow 1969-1970  
Chief Medical Resident 1970-1971  
Post-doctoral Fellow 1971-1972  
Laboratory of R. T. Prehn, M.D.  
The Institute for Cancer Research  
7701 Burholme Avenue  
Philadelphia, PA 19111

## MILITARY SERVICE

CPT, MC, U.S. Army, Molecular Biology Branch, 1965-1967  
Medical Laboratories, Edgewood Arsenal, MD

## SPECIALTY CERTIFICATION

Diplomate of the American Board of Internal Medicine 1972  
Certificate #37358  
Diplomate, subspecialty of Medical Oncology 1973  
Certificate #37358

## LICENSURE

Maryland - D7736 1964  
Pennsylvania - MD 008521-E 1965  
New Jersey - MA24857 1971

## HONORARY SOCIETIES

Delta Epsilon Sigma 1960  
National Collegiate Who's Who 1960  
Sigmi Xi 1984

# PROFESSIONAL SOCIETIES

College of Physicians of Philadelphia	1971
Philadelphia County Medical Society	1972-1986
Pennsylvania Medical Society	1972-1986
American Medical Association	1972-1986
American Federation for Clinical Research	1972
American Association for the Advancement of Science	1973
American Society of Clinical Oncology	1973
American College of Physicians - Member	1973
- Fellow	1975
American College of Clinical Pharmacology Fellow	1974
American Association for Cancer Research	1974
Program Committee for Immunology - Member	1979-1980
" " " " - Chairperson	1987-1988
Membership Committee	1984-1985
Gertrude Elion Award Committee	1993-1994
Society for Biological Therapy	1981-
Board of Directors	1988-1991
Chairman, Program and Publications Committee	1989-1990
President	1990-1992

## PROFESSIONAL ACTIVITIES

Seminars in Oncology - Associate Editor	1974-
Eastern Cooperative Oncology Group - Member	1973-1976
Malignant Melanoma Clinical Cooperative Group - Member	1973-1977
Committee on Tumor Immunotherapy, DCBD, NCI - Member	1975-1979
American Cancer Society, Philadelphia Division	
Member, Professional Education Committee	1975-1977
Member, Board, Northeast Unit	1978-1979
American Cancer Society, National Office	
Member, Advisory Committee on Immunology and Immunotherapy	1987-1991
Philadelphia County Medical Society -	1976-1984
Member, Cancer Control Subcommittee	1976-1977
Experimental Therapeutic Study Section, NIH -	
Member	1980-1982
Chairman I + II	1982-1984
Cancer Immunology and Immunotherapy - Board of Editors	1981-1993
Subcommittee on Biological Response Modifiers of the	1978-1980
Division of Cancer Treatment's Board of Scientific Counsellors	
Biological Response Modifier Program, DCT, NCI -	
Operating Committee, Member	1980-1982
Decision Network Committee, Member	1980-1984
Journal of Biological Response Modifiers - Editorial Board	1982-1990
Journal of Immunotherapy - Editorial Board	1990-
Cancer Research - Associate Editor	1983-1994
Hybridoma - Editorial Board	1987-1992
PDQ - Extramural Board	1989-1993
The United States Pharmacopeial Convention, Inc.	
Advisory Panel on Hematologic and Neoplastic Diseases	1990-1995
Vaccine Research - Associate Editor, Tumor Vaccines	1990-

## FACULTY AND APPOINTMENTS

## Current -

Professor of Medicine,	1984-
Director, Division Medical Oncology	1984-1993
Member, Division Neoplastic Diseases	1993-
Jefferson Medical College	
1025 Walnut Street	
Philadelphia, PA 19107	

Courtesy Staff	1987-
Associate Staff	1984-1987
Associate Physician (Medicine)	1977-1984
Assistant Physician (Medicine)	1972-1976
Director, Pigmented Lesion Clinic	1972-1984
American Oncologic Hospital	
Central and Shelmire Avenues	
Philadelphia, PA	

Consultant, Oncology	1973-
Department of Medicine	
The Mercer Medical Center	
Trenton, NJ	

Consultant, Oncology Division	1974-
Retina Service	
Wills Eyes Hospital	
Philadelphia, PA	

## Prior

Instructor (Medicine)	1970-1974
Assistant Physician (Medicine)	1971-1974
Jefferson Medical College and Hospital	
Philadelphia, PA 19107	

Clinical Associate Professor (Medicine)	1977-1984
Clinical Assistant Professor (Medicine)	1974-1977
School of Medicine	
Temple University Health Science Center	
Philadelphia, PA 19107	

Research Physician	1972-1980
The Institute for Cancer Research	
Philadelphia, PA	

Consultant, Medical Oncology	1976-1986
Doylestown Hospital	
Doylestown, PA	

Consultant, Tumor Immunotherapy	1973-1988
Chestnut Hill Hospital	
Philadelphia, PA	



PUBLICATIONSAPERS

1. Mastrangelo, M.J., Giordano, W.P. and Johnson, R.P. Surface behavior of individual lipids similar to constituents of pulmonary surfactant. EATR 4087, April 1967.
2. Mastrangelo, M.J. and Johnson, R.P. Segmental reversibility and hysteresis of preparations similar to pulmonary surfactant. EATR 4097, May 1967.
3. Mastrangelo, M.J., Carwile, H. and Johnson, R.P. Qualitative protein composition of lung surfactant preparations. EATR 4124, August 1967.
- \*4. Weiss, A.J. and Mastrangelo, M.J. Phase I study of a combination of azotomycin (NSC-56654) and 5-Fluorouracil (NSC-19893) in malignant disease. Cancer Chemother Rpt. 54:109-112, 1970.
5. Mastrangelo, M.J. and Weiss, A.J. The chemotherapy of respiratory tract neoplasms. In: Cancer Chemotherapy II, I. Brodsky, S.B. Kahn, J.H. Moyer (Editors), Grune and Stratton, Inc., New York, NY, 1972, pp. 195-208.
- \*6. Weiss, A.J., Stambaugh, J.E., Mastrangelo, M.J., Laucius, J.F. and Bellet, R.E. A Phase I study of 5-azacytidine (NSC-102816). Cancer Chemother. Rpt. 56:413-420, 1972.
7. Mastrangelo, M.J., Sulit, H.O., Chee, D. and Engstrom, P.F. Cancer Forum: Malignant Melanoma. Penna. Med. 75:43, 1972.
- \*8. Mastrangelo, M.J., Grage, T. Bellet, R.E. and Weiss, A.J. A Phase I study of emetine hydrochloride (NSC-33669) in solid tumors. Cancer 31:1170-1175, 1973.
- \*9. Bellet, R.E., Mastrangelo, M.J., Engstrom, P.F. and Custer, R.P. Hepatotoxicity of 5-azacytidine (NSC-102816): A clinical and pathologic study. Neoplasma 20:303-310, 1973.
- \*10. Bellet, R.E., Mastrangelo, M.J., Dixon, L.M. and Yarbrow, J.W. A Phase I study of ICRF 159 (NSC-129943) in solid tumors. Cancer Chemother. Rpt. 57:185-189, 1973.
11. Mastrangelo, M.J., Creech, R.H. and Engstrom, P.F. Cancer Forum: Early diagnosis holds promise in lung cancer. Penna. Med. 76:64, 1973.
12. Bornstein, R.S., Mastrangelo, M.J., Sulit, H.L., Chee, D.O., Yarbrow, J.W., Prehn, L.M. and Prehn, R.T. Immunotherapy of melanoma with intralesional BCG. Natl. Cancer Inst. Monogr. 39:213-220, 1973.
- \*13. Mastrangelo, M.J., Kim, Y.H., Bornstein, R.S., Chee, D.O., Sulit, H.L., Yarbrow, J.W. and Prehn, R.T. Clinical and histological correlation of melanoma regression following intralesional BCG therapy: A case report. J. Natl. Cancer Inst. 52:19-24, 1974.
- \*14. Bellet, R.E., Mastrangelo, M.J., Engstrom, P.F., Strawitz, J.G. and Weiss, A.J. Clinical trial with subcutaneously administered 5-Azacytidine (NSC-102816). Cancer Chemother. Rpt. 58:217-222, 1974.

- \*15. Bodurtha, A., Kim, Y.H., Laucius, J.F., Donato, R.A. and Mastrangelo, M.J. Hepatic granulomas and other hepatic lesions associated with BCG immunotherapy for cancer. *Am. J. Clin. Pathol.* 61:747-752, 1974.
- \*16. Bellet, R.E., Mastrangelo, M.J. and Dixon, L.M. Letter to the Editor: ICRF 159. *Lancet* 1:926, 1974.
- 17. Mastrangelo, M.J., Laucius, J.F. and Outzen, H.C. Fundamental concepts in tumor immunology: A brief review. *Semin. Oncol.* 1:291-296, 1974.
- \*18. Laucius, J.F., Bodurtha, A.J., Mastrangelo, M.J. and Creech, R.H. Bacillus Calmette-Guerin in the treatment of neoplastic disease. *J. Reticuloendothel. Soc.* 16:347-373, 1974. (Abstracted Year Book of Cancer 1976).
- \*19. Bodurtha, A.J., Chee, D.O., Laucius, J.F., Mastrangelo, M.J. and Prehn, R.T. Clinical and immunological significance of human melanoma cytotoxic antibody. *Cancer Res.* 38:189-193, 1975.
- \*20. Creech, R.H., Catalano, R.B., Mastrangelo, M.J. and Engstrom, P.F. Low dose cyclophosphamide, methotrexate, and 5-fluorouracil therapy of metastatic breast cancer. *Cancer* 35:1101-1107, 1975.
- \*21. Mastrangelo, M.J., Bellet, R.E., Berkelhammer, J. and Clark, W.H., Jr. Regression of pulmonary metastatic disease associated with intralesional BCG therapy of dermal melanoma metastases. *Cancer* 36:1305-1308, 1975. (Abstracted Year Book of Diagnostic Radiology 1977).
- \*22. Berkelhammer, J., Mastrangelo, M.J., Laucius, J.F., Bodurtha, A.J. and Prehn, R.T. Sequential in vitro reactivity of lymphocytes from melanoma patients receiving immunotherapy compared with the reactivity of lymphocytes from healthy donors. *Int. J. Cancer* 16:571-578, 1975.
- \*23. Sieper, W.J., Mastrangelo, M.J. and Bellet, R.E. Phase II study of adriamycin (NSC-123127) in patients with metastatic melanoma. *Cancer Chemother. Rpt.* 59:1181-1182, 1975.
- \*24. Pliskin, M.E., Mastrangelo, M.J., Brown, A.M. and Custer, R.P. Melanoma of the maxilla presenting as a gingival swelling. *Oral Surg. Oral Med. Oral Pathol.* 41:101-104, 1976.
- \*25. Mastrangelo, M.J., Sulit, H.L., Prehn, L.M., Bornstein, R.S., Yarbrow, J.W. and Prehn, R.T. Intralesional BCG in the treatment of metastatic malignant melanoma. *Cancer* 37:684-692, 1976.
- \*26. Bodurtha, A.J., Berkelhammer, J., Kim, Y.H., Laucius, J.F. and Mastrangelo, M.J. A clinical, histological and immunological study of a case of metastatic malignant melanoma undergoing spontaneous remission. *Cancer* 37:735-742, 1976.
- \*27. Ainsworth, A.M., Clark, W.H., Jr., Mastrangelo, M.J. and Conger, K.B. Primary malignant melanoma of the urinary bladder. *Cancer* 37:1928-1936, 1976.

28. Bellet, R.E. and Mastrangelo, M.J. Investigational new drugs. In: Oncologic Medicine, P.F. Engstrom, A.I. Sutnick (Editors), University Park Press, Baltimore, MD, 1976, pp. 237-245.
- \*29. Mastrangelo, M.J., Bellet, R.E., Laucius, J.F. and Berkelhammer, J. Immunotherapy of malignant melanoma. A Review. In: Oncologic Medicine, P.F. Engstrom, A.I. Sutnick (Editors), University Park Press, Baltimore, MD, 1976, pp. 71-93.
- \*30. Pliskin, M.E., Mastrangelo, M.J., Bellet, R.E. and Berkelhammer, J. BCG immunotherapy of a mucous membrane malignant melanoma (a case report). Oral Surg. Oral Med. Oral Pathol. 42:73-79, 1976.
- \*31. Mastrangelo, M.J., Berd, D. and Bellet, R.E. Critical review of previously reported clinical trials of cancer immunotherapy with non-specific immunostimulants. Ann. N.Y. Acad. Sci. 277:94-123, 1976.
- \*32. Bellet, R.E., Mastrangelo, M.J., Laucius, J.F. and Bodurtha, A.J. A randomized prospective trial of DTIC (NSC-45388) alone versus BCNU (NSC-409962) plus vincristine (NSC-67574) in the treatment of metastatic malignant melanoma. Cancer Treat. Rpt. 60:595-600, 1976.
33. Mastrangelo, M.J., Berd, D. and Bellet, R.E. Review of immunotherapeutic studies on cancer patients. In: Immuno-Cancerology in Solid Tumors, M. Martin, L. Dionne (Editors), Stratton Intercontinental Medical Book Corporation, New York, NY, 1976, pp. 155-170.
- \*34. Bellet, R.E., Catalano, R.B., Danna, V.G., Berkelhammer, J. and Mastrangelo, M.J. A study of antitumor (Phase II) and immunosuppressive effects of ICRF-159 (NSC-129943) in patients with metastatic melanoma. J. Clin. Pharm. 16:433-438, 1976.
- \*35. Bellet, R.E., Engstrom, P.F., Catalano, R.B., Creech, R.H. and Mastrangelo, M.J. Phase II study of ICRF-159 (NSC-129943) in patients with metastatic colorectal carcinoma previously exposed to systemic chemotherapy. Cancer Treat. Rpt. 60:1395-1397, 1976.
36. Clark, W.H., Jr., Mastrangelo, M.J., Ainsworth, A.M., Berd, D., Bellet, R.E. and Bernardino, E.A. Current concepts of the biology of human cutaneous malignant melanoma. Adv. Cancer Res. 24:267-338, 1977.
- \*37. Berkelhammer, J., Mastrangelo, M.J. and Prehn, R.T. Effect of treatment with dimethyl triazeno imidazole carboxamide (DTIC, NSC-45388) or 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, NSC-409962) plus vincristine (NSC-67564) on lymphocyte reactivity of melanoma patients. Cancer Immunol. Immunother. 2:119-125, 1977.
- \*38. Laucius, J.F., Patel, Y.A., Koons, L.S., Bellet, R.E. and Mastrangelo, M.J. A Phase II evaluation of Bacillus Calmette-Guerin plus megestrol acetate in patients with metastatic renal adenocarcinoma. Med. Pediatr. Oncol. 3:237-242, 1977.
- \*39. Bellet, R.E., Vaisman, I., Mastrangelo, M.J. and Lustbader, E. Multiple primary malignancies in patients with cutaneous melanoma. Cancer 40:1974-1981, 1977.

- \*40. Laucius, J.F., Bodurtha, A.J., Mastrangelo, M.J. and Bellet, R.E. A Phase II study of autologous irradiated tumor cells plus BCG in patients with metastatic malignant melanoma. *Cancer* 40:2091-2093, 1977.
41. Mastrangelo, M.J., Bellet, R.E., Berd, D., and Lustbader, E. A randomized prospective trial comparing methyl-CCNU + vincristine to methyl-CCNU + vincristine + BCG + allogeneic tumor cells in patients with metastatic malignant melanoma. In: *Immunotherapy of Cancer: Current Status of Trials in Man*, W. Terry and D. Windhorst (Editors), Progress in Cancer Research and Therapy, Vol. 6, Raven Press, New York, NY, 1978, pp. 95-102.
42. Mastrangelo, M.J., Clark, W.H., Jr., Bellet, R.E. and Berd, D. Cutaneous malignant melanoma: Diagnosis prognosis and conventional therapy. In: *Immunotherapy of Cancer: Current Status of Trials in Man*, W. Terry, D. Windhorst (Editors), Progress in Cancer Research and Therapy, Vol. 6, Raven Press, New York, NY, 1978, pp. 1-17.
43. Engstrom, P.F., Paul, A.R., Catalano, R.B., Mastrangelo, M.J. and Creech, R.H. Fluorouracil versus fluorouracil + BCG in colorectal adenocarcinoma. In: *Immunotherapy of Cancer: Current Status of Trials in Man*, W. Terry, D. Windhorst (Editors), Progress in Cancer Research and Therapy, Vol. 6, Raven Press, New York, NY, 1978, pp. 587-596.
- \*44. Bellet, R.E., Catalano, R.B., Mastrangelo, M.J. and Berd, D. Phase II study of subcutaneously administered 5-azacytidine (NSC-102816) in patients with metastatic malignant melanoma. *Med. Pediatr. Oncol.* 4:11-15, 1978.
45. Greene, M.H., Reimer, R.R., Clark, W.H., Jr. and Mastrangelo, M.J. Precursor lesions in familial melanoma. *Semin. Oncol.* 5:85-87, 1978.
- \*46. Bellet, R.E., Catalano, R.B., Mastrangelo, M.J., Berd, D. and Koons, L.S. Phase II trial of VM-26 (NSC-122819) in patients with metastatic melanoma. *Cancer Treat. Rpt.* 62:445-447, 1978.
- \*47. Clark, W.H., Jr., Reimer, R.R., Green, M., Ainsworth, A.M. and Mastrangelo, M.J. Origin of familial malignant melanoma from heritable melanocytic lesions. The B-K mole syndrome. *Arch. Derm.* 114:732-738, 1978.
48. Berkelhammer, J., Mastrangelo, M.J., Bellet, R.E., Prehn, R.T. and Thibault, L.H. Failure of lymphocyte microcytotoxicity to distinguish relapsers from non-relapsers in melanoma patients receiving postsurgical adjuvant chemotherapy. *Eur. J. Cancer* 14:793-798, 1978.
49. Mastrangelo, M.J., Berd, D. and Bellet, R.E. Limitations, obstacles and controversies in the optimal development of immunotherapy. In: *Immunotherapy of Human Cancer*, E. Herish, J. Sinkovics (Editors), Raven Press, New York, NY, 1978, pp. 375-394.
50. Bellet, R.E., Mastrangelo, M.J., Berd, D. and Lustbader, E. Melanoma chemotherapy. In: *Cancer Chemotherapy III*, I Brodsky, S.B. Kahn, J.F. Conroy (Editors), Grune and Stratton, Inc., New York, 1978, pp. 225-242.
- \*51. Mastrangelo, M.J. and Bellet, R.E. Immunotherapy of melanoma. In: *Clinical Therapeutics*, D.T. Lowenthal, D.A. Major II, (Editors), Grune and Stratton, Inc., New York, 1978, pp. 281-291.

- \*52. Bellet, R.E., Catalano, R.B., Mastrangelo, M.J. and Berd, D. A positive Phase II trial of dibromodulcitol in patients with metastatic melanoma refractory to DTIC and nitrosourea. *Cancer Treat. Rpt.* 62:2095-2099, 1978.
53. Bellet, R.E., Mastrangelo, M.J. and Berd, D. Chemotherapy of metastatic malignant melanoma. *In: Human Malignant Melanoma*, Grune and Stratton, Inc., New York, 1979, pp. 355-416.
54. Vaisman, I., Mastrangelo, M.J., Bellet, R.E. and Lustbader, E. Primary cutaneous melanoma and coexisting malignant neoplasms. *In: Human Malignant Melanoma*, Grune and Stratton, Inc., New York, 1979, pp. 243-260.
55. Mastrangelo, M.J., Bellet, R.E. and Berd, D. Immunology and immunotherapy of malignant melanoma. *In: Human Malignant Melanoma*, Grune and Stratton, Inc., New York, 1979, pp. 355-416.
56. Mastrangelo, M.J., Bellet, R.E. and Berd, D. Adjuvant therapy. *In: Human Malignant Melanoma*, Grune and Stratton, Inc., New York, 1979, pp. 309-324.
57. Mastrangelo, M.J., Bellet, R.E. and Berd, D. Prognostic Factors. *In: Human Malignant Melanoma*, Grune and Stratton, Inc., New York, 1979, pp. 273-281.
58. Berd, D., Mastrangelo, M.J. and Bellet, R.E. Metastatic uveal melanoma. *In: Human Malignant Melanoma*, Grune and Stratton, Inc., New York, 1979, pp. 489-496.
59. Laucius, J.F. and Mastrangelo, M.J. Cutaneous depigmentary phenomena in patients with malignant melanoma. *In: Human Malignant Melanoma*, Grune and Stratton, Inc., New York, 1979, pp. 209-226.
- \*60. Berkelhammer, J., Mastrangelo, M.J., Bellet, R.E., Berd, D. and Prehn, R.T. Chemoimmunotherapy increases the lymphocyte reactivity of melanoma patients. *Eur. J. Cancer* 15:197-204, 1979.
- \*61. Mastrangelo, M.J., Bellet, R.E. and Berd, D. A Phase III comparison of methyl-CCNU + vincristine with or without BCG + allogeneic tumor cells in metastatic melanoma. *Cancer Immunol. Immunother.* 6:231-236, 1979.
- \*62. Goepp, C.E., Mastrangelo, M.J., Patel, Y.A. and Clark, W.H., Jr. Cutaneous malignant melanoma in a patient with neurofibromatosis. *Arch. Dermatol.* 115:364-366, 1979.
- \*63. Berd, D., Wilson, E.J., Bellet, R.E. and Mastrangelo, M.J. The effect of methyl-CCNU adjuvant therapy on immune responses of patients with malignant melanoma. *Cancer Res.* 39:4472-4476, 1979.
- \*64. Bellet, R.E., Danna, V., Mastrangelo, M.J. and Berd, D. Evaluation of a "nude" mouse-human tumor panel as a predictive secondary screen for cancer chemotherapeutic agents. *J. Natl. Cancer Inst.* 63:1185-1188, 1979.
65. Tems-Reines, S.J. and Mastrangelo, M.J. *In: Clinical Immunotherapy*, A. F. LoBuglio (Editor), Marcel Dekker, Inc., NY, 1980, pp. 7-29.

- \*66. Augsburger, J.J., Shields, J.A., Mastrangelo, M.J. and Frank, P.F. Diffuse primary malignant melanoma of the choroid arising in a patient with prior cutaneous malignant melanoma. *Arch. Ophthalmol.* 98:1261-1264, 1980.
- \*67. Herlyn, M., Clark, W.H., Jr., Mastrangelo, M.J., Guerry, D. IV, Elder, D.E., LaRossa, D., Hamilton, R., Bondi, E.M., Tuthill, R., Steplewski, Z. and Koprowski, H. Specific immunoreactivity of monoclonal anti-melanoma antibodies. *Cancer Res.* 40:3602-3609, 1980.
68. Mastrangelo, M.J., Hersh, E. and Chirigos, M. Augmenting agents in cancer therapy: Workshop summation. *In: Augmenting Agents in Cancer*, E. Hersh, M. Chirigos, M. Mastrangelo (Editors), Raven Press, New York, NY, 1981, pp. 553-564.
- \*69. Berd, D. and Mastrangelo, M.J. Differential sensitivity of two murine leukemia sublines to cytolysis by Corynebacterium parvum-activated macrophages. *Br. J. Cancer* 44:819-827, 1981.
70. Bellet, R.E. and Mastrangelo, M.J. Malignant melanoma: Investigations in the "nude mouse." *In: The Nude Mouse in Experimental and Clinical Research*, Vol. II, J. Fogh, B. Giovanella (Editors), Academic Press, New York, NY, 1982, pp. 511-519.
71. Chirigos, M. and Mastrangelo, M.J. Immunorestitution by chemicals. *In: Immunologic Aspects of Cancer Therapeutics*, E. Mihich (Editor), John Wiley and Sons, Inc., NY, 1982, pp. 191-239.
72. Mastrangelo, M.J., Rosenberg, S.A., Baker, A. and Katz, H. Cutaneous melanoma. *In: Cancer Principles and Practices of Oncology*, V.T. DeVita, S. Hellerman, S. Rosenberg (Editors), J.B. Lippincott, Co., Philadelphia, PA, 1982, pp. 1124-1130.
73. Mastrangelo, M.J. and Berd, D. Immunotherapy with microbial products. *In: Immunological Aspects of Cancer Therapeutics*, E. Mihich (Editor), John Wiley and Sons, Inc., NY, 1982, pp. 75-105.
- \*74. Berd, D., Mastrangelo, M.J., Engstrom, P.F., Paul, A. and Maguire, H. Augmentation of the human immune response by cyclophosphamide. *Cancer Res.* 42:4862-4866, 1982.
75. Bellet, R.E., Mastrangelo, M.J., Berd, D. and Maguire, H.C. Primary cutaneous melanoma. *In: Concepts in Cancer Medicine*, S.B. Kahn, R. Love, R. Chakravorty, C.W. Sherman (Editors), Grune and Stratton, Inc., New York, 1982, Chapter 9, pp. 545-555.
76. Bellet, R.E., Danna, V., Mastrangelo, M.J., Eaton, G.J. and Berd, D. Evaluation of the response of a panel of human melanoma tissue-cultured cell lines xenografted in nude mice to four anticancer drugs of known clinical activity. *Proc. 3rd Int. Workshop on Nude Mice*, Gustav Fischer, New York, NY, 1982, pp. 649-656.
- \*77. Donos, L.A., Nagy, R.M., Brockman, R.J., Augsburger, J.J., Shields, J.A., Berd, D. and Mastrangelo, M.J. Metastatic uveal melanoma. Hepatic cell-surface enzymes, isoenzymes and serum sialic acid levels in early metastatic disease. *Arch. Ophthalmol.* 101:791-794, 1983.

- \*78. Berd, D., Maguire, H.C. and Mastrangelo, M.J. Impairment of concanavalin A-inducible suppressor activity following administration of cyclophosphamide to patients with advanced cancer. *Cancer Res.* 44:1275-1280, 1984.
- \*79. Mastrangelo, M.J., Berd, D. and Maguire, H.C. The current condition and prognosis of tumor immunotherapy: A second opinion. *Cancer Treat. Rpt.* 68:207-210, 1984.
- 80. Berd, D., Maguire, H.C. and Mastrangelo, M.J. Immunopotential by cyclophosphamide and other cytotoxic agents. In: *Immunomodulating Agents*, R. Fenichel (Editor), Marcel Dekker, 1984, pp 39-62.
- 81. Berd, D., Maguire, H.C. and Mastrangelo, M.J. Potentiation of human cell-mediated and humoral immunity by low dose cyclophosphamide. *Cancer Res.* 44:5439-1984.
- \*82. Donoso, L.A., Berd, D., Augsburger, J.J., Mastrangelo, M.J. and Shields, Metastatic uveal melanoma: Pretherapy serum liver enzyme and liver scan abnormalities. *Arch. Ophthalmol.* 103:796-798, 1985.
- \*83. Goldman, L.I., Elder, D.E., Mastrangelo, M.J., Clark, W.H. and Stennett, J. Assessment of survival with metastatic malignant melanomas. *Surg. Gynecol. Obstet.* 162:199-203, 1986.
- \*84. Berd, D., Maguire, H.C. and Mastrangelo, M.J. Induction of cell-mediated immunity to autologous melanoma cells and regression of metastases after treatment with a melanoma cell vaccine preceded by cyclophosphamide. *Cancer Res.* 46:2572-2577, 1986.
- 85. Mastrangelo, M.J., Berd, D. and Maguire, H., Jr. The immunoaugmenting effects of cancer chemotherapeutic agents. *Semin. Oncol.* 13:186-194, 1986.
- \*86. Slovin, S.F., Lackman, R.D., Ferrone, S., Kiely, P.E. and Mastrangelo, M.J. Cellular immune response to human sarcomas: Cytotoxic T cell clones reactive with autologous sarcomas. I. Development, phenotype and specificity. *J. Immunol.* 137:3042-3048, 1986.
- \*87. McClay, E., Lusch, C.J. and Mastrangelo, M.J. Allergy induced hepatic toxicity associated with DTIC (Dacarbazine). *Cancer Treat. Rep.* 71:219-220, 1987.
- \*88. McClay, E., Mastrangelo, M.J., Bellet, R.E. and Berd, D. Combination chemo/hormonal therapy in the treatment of malignant melanoma. *Cancer Treat. Rep.* 71:465-469, 1987.
- \*89. Slovin, S.F., Lackman, R.D., Mastrangelo, M.J. and the Thomas Jefferson University Sarcoma Study Group. HLA-A2 in sarcomas: An immunologic perspective. *Transplantation* 43:923-925, 1987.
- \*90. Berd, D. and Mastrangelo, M.J. Depletion of suppressor T lymphocytes by administration of a murine monoclonal antibody. *Cancer Res.* 47:2727-2732, 1987.
- \*91. Mastrangelo, M.J. and Berd, D. Control of disseminated melanoma with intralesional immunotherapy. In: *Cutaneous Melanoma. Status of Knowledge and Future Perspective* U. Veronesi, N. Cascinelli, M. Santinami (Editors), Academic Press, 1987, pp. 643-648.

- \*92. Berd, D. and Mastrangelo, M.J. Effect of low dose cyclophosphamide on the immune system of cancer patients: Reduction of T suppressor function without depletion of the CD8+ subset. *Cancer Res.* 47:3317-3321, 1987. (Abstracted Year Book of Cancer 1988).
- \*93. Berd, D. and Mastrangelo, M.J. Elimination of immune suppressor mechanisms in humans by oxazaphosphorines. *Meth. Find. Exp. Clin. Pharmacol.* 9:569-577, 1987.
- \*94. Berd, D. and Mastrangelo, M.J. Effect of low dose cyclophosphamide on the immune system of cancer patients: Depletion of CD4+ 2H4+ suppressor-inducer T cells. *Cancer Res.* 48:1671-1675, 1988.
- \*95. Berd, D. and Mastrangelo, M.J. Active immunotherapy of human melanoma exploiting the immunopotentiating effects of cyclophosphamide. *Cancer Investigation* 337-349, 1988.
- \*96. McClay, E.J., Mastrangelo, M.J., Berd, D. and Bellet, R.E. Letter to the Editor: Hypercoagulable state secondary to tumor lysis. *N. Engl. J. Med.* 319:440, 1988.
- 97. McClay, E.J. and Mastrangelo, M.J. Systemic chemotherapy for metastatic melanoma. *Semin. Oncol.* 15:569-577, 1988.
- 98. Mastrangelo, M.J., Schultz, S., Kane, M. and Berd, D. Newer immunologic approaches to the treatment of patients with melanoma. *Semin. Oncol.* 15:578-588, 1988.
- 99. Mastrangelo, M.J. Recent advances in the medical management of metastatic melanoma. *Mediguide Oncol.* 9:1-6, 1989.
- 100. Schultz, S. and Mastrangelo, M.J. The pathophysiology and etiology of malignant melanoma. *Semin. Oncol.* 16 (Suppl 1):27-33, 1989.
- 101. Harris, D.T. and Mastrangelo, M.J. Serotherapy of cancer. *Semin. Oncol.* 16:180-198, 1989.
- \*102. McClay, E.J., Mastrangelo, M.J., Sprandio, J.D., Bellet, R.E., and Berd, D. The importance of tamoxifen to a cisplatin containing regimen in the treatment of metastatic melanoma. *Cancer* 63:1292-1295, 1989.
- \*103. Ehrke, M.J., Mihich, E., Berd, D. and Mastrangelo, M.J. Effects of anti-cancer drugs on the immune system in man. *Semin. Oncol.* 16:230-253, 1989.
- \*104. Turner, B.J., Siatowski, R.M., Augsburger, J.J., Shields, J.A., Lustbader, E. and Mastrangelo, M.J. Cancers in uveal melanoma patients and their families: Implication for the genesis of this tumor. *Am. J. Ophthal.* 107:601-603, 1989.
- 105. Mastrangelo, M.J. and Berd, D. Immunoregulation and active specific immunization in cancer treatment. In: *Human Melanoma - Immunology, Diagnostics and Therapy.* S. Ferrone (Editor), Springer Verlag, New York, 1989, pp. 497-512.
- \*106. Berd, D., Herlyn, M., Koprowski, H. and Mastrangelo, M.J. Flow cytometric determination of the frequency and heterogeneity of expression of human melanoma-associated antigens. *Cancer Res.* 49:6840-6844, 1989.



- \*107. Cardi G., Mastrangelo, M.J. and Berd, D. Depletion of T cells with the CD4+CD45R + phenotype in lymphocytes that infiltrate subcutaneous metastases of human melanoma. *Cancer Res.* 49:6562-6565, 1989.
- \*108. Schultz, S., Kane, M., Roush, R., Miller, V., Berd, D., Goldman, L. and Mastrangelo, M.J. Time to recurrence varies inversely with thickness in clinical Stage I cutaneous melanoma. *Surg. Gynecol. Obstet.* 171:393-397, 1990.
- \*109. Berd, D., Maguire, H.C., Jr., Mc Cue, P. and Mastrangelo, M.J. Immunotherapy of metastatic melanoma with an autologous tumor cell vaccine: Clinical and Immunological results in 64 patients. *J. Clin. Oncol.* 8:1858-1867, 1990.
- \*110. McLean, I.W., Berd, D., Mastrangelo, M.J., Shields, J.A., Davidorf, F.H., Grever, M., Makley, T.A. and Gamel, J.W. A randomized study of methanol extraction residue of Bacille Calmette-Guerin as postsurgical adjuvant therapy of uveal melanoma. *Am. J. Ophthalm.* 110:522-526, 1990.
- \*111. Slovin, S.F., Maguire, H.C., Jr., Lackman, R.D. and Mastrangelo, M.J. The role of autologous tumor cells in preventing lymphokine-activated killer cell induction in vitro. *Cancer* 66:2541-2546, 1990.
- 112. Mastrangelo, M.J., Bellet, R.E. and Berd D. Aggressive chemotherapy for melanoma. *PPO Updates* 5 (May):1-11, 1991.
- 113. Harris, D.T. and Mastrangelo, M.J.: Theory and application of early systemic therapy. *Semin. Oncol.* 18:493-503, 1991.
- \*114. Berd, D., Murphy, G., Maguire, H.C., Jr. and Mastrangelo, M.J. Immunization with hapteneized, autologous tumor cells induces inflammation of human melanoma metastases. *Cancer Res* 51:2731-2734, 1991.
- \*115. Herlyn, D., Benden, A., Kane, M., Somasundaram, R., Zaloudik, J., Sperlagh, M., Marks, G., Hart, E., Ralph C., Wettendorff, M. and Mastrangelo, M.J. Anti-idiotypic cancer vaccines: Preclinical and clinical studies. *In Vivo* 5:615-624, 1991.
- 116. Mastrangelo, M.J., Bellet, R.E., Kane, M.J. and Berd D. Melanoma. In: Chemotherapy Source Book, M. Perry (Editor), William and Wilkins, Baltimore 1992, pp 886-907.
- \*117. McClay, E.F., Mastrangelo, M.J., Berd, D., and Bellet, R.E. Effective combination chemo/hormonal therapy for malignant melanoma: Experience with three consecutive trials. *Int J Cancer* 50:553-556, 1992.
- \*118. Fishbein, G.E., McClay, E., Berd, D., Mastrangelo, M.J.: A post surgical adjuvant trial of CEA immunization in patients with Dukes C and D colorectal cancer. A pilot study. *Vaccine Res* 1:123-128, 1992.
- 119. Mastrangelo, M.J., Harris, D.T.: Cutaneous Melanoma. In: Current Therapy in Oncology. J. Niederhuber (Editor), B. C. Decker, St. Louis, 1993, pp 132-138

120. McLaughlin, C., Maguire, H.C. Jr. and Mastrangelo, M.J. Case Report: The natural history of a superficial spreading melanoma. *Int. J. Dermatol.* 32:293-296, 1993.
- \*121. Berd, D., Maguire, H.C. Jr., Mastrangelo, M.J. Treatment of human melanoma with a hapten-modified autologous vaccine. *Ann. N.Y. Acad. Sci.* 690:147-152, 1993.
- \*122. Herlyn, D., Zaloudik, J., Somasundaram, R., Jacob, L., Benden, A. and Mastrangelo, M.J. Anti-idiotypic vaccine in colorectal cancer patients. *Hybridoma* 12:515-520, 1993.
123. Mastrangelo, M.J. Controlled studies in chemotherapy for advanced melanoma. In: *Malignant Melanoma: The approach of the surgical oncologist*, F.J. Lejeune, P.K. Chaudhuri, T.K. Das Gupta (Editors), McGraw-Hill, Inc., New York, NY 1994, pp. 295-301.
124. Herlyn, D., Harris, D., Zaloudik, J., Sperlagh, M., Maruyama, H., Jacob, L., Kieny, M-P, Scheck, S., Somasundaram, R., Hart, E., Erte, H. and Mastrangelo, M.J. Immunomodulatory activity of monoclonal anti-idiotypic antibody to anti-colorectal carcinoma antibody CO17-1A in animals and in patients. *J. Immunother* (accepted 1/94).
- \*125. Berd, D., Maguire, H.C., Jr., Mastrangelo, M.J. and Murphy, G.P. Activation markers on T cells infiltrating melanoma metastases after therapy with dinitrophenyl conjugated vaccine (submitted 8/93).
- \*126. Somasundaram, R., Zaloudik, J., Jacob, L., Benden, A., Sperlagh, M., Hart, E., Marks, G., Kane, M., Mastrangelo, M., Herlyn, D. Induction of B- and T-cell immunity in colon cancer patients by anti-idiotypic antibody. (Submitted 2/93).
- \*127. Sato, T., Maguire, H.C., Jr., Mastrangelo, M.J. and Berd, D. Human immune response to DNP-modified autologous cells after treatment with a DNP-conjugated vaccine. *J Immunol* (submitted 2/94).
- \*128. Lee, S.S., Eisenlohr, L.C., McCue, P.A., Mastrangelo, M.J. and Lattime, E.C. Intravesical gene therapy: In-vivo gene transfer using recombinant vaccinia virus vectors. *Cancer Research* (submitted 2/94).
129. Mastrangelo, M.J., Maguire H.C., Jr., Lattime, E.C. and Berd, D. Whole cell vaccines. In: *Biological Therapy of Cancer*, 2nd Edition, DeVita, V.T., Hellman, S., Rosenberg, S.A. (Editors), Lippincott, Philadelphia (submitted 2/94).
- \*130. Berd, D., Wiebe, V., Mastrangelo, M.J., Bellet, R.E. and DeGregorio, M.W. Short course, high dose tamoxifen with cytotoxic chemotherapy for metastatic melanomas. *Int J Cancer* (submitted 9/91).

# ABSTRACTS

1. Mastrangelo, M.J., Giordano, W.P. and Johnson, R.P. Surface behavior of individual lipids similar to pulmonary surfactant. *Physiologist* 9:240, 1966.
- A2. Mastrangelo, M.J., Bornstein, R.S. and Yarbrow, J.W. A clinical trial of adriamycin in adult solid tumors. *Clin. Res.* 19:738, 1971.
- A3. Mastrangelo, M.J., Bornstein, R.S., Sulit, H.L., Chee, D.O., Yarbrow, J.W. and Prehn, R.T. Immunotherapy of malignant. *Ann. Intern. Med.* 76:877-878, 1972 (Abstract #80).
- A4. Sulit, H.L., Chee, D.O., Mastrangelo, M.J., Engstrom, P.F., Bornstein, R.S., Prehn, R.T. and Yarbrow, J.W. In vitro assays of cellular and humoral immunity during clinical trials of immunotherapy in human melanoma. *Proc. Am. Assoc. Cancer Res.* 13:74, 1972 (Abstract #294).
- A5. Bellet, R.E., Mastrangelo, M.J., Engstrom, P.F., Bornstein, R.S., Strawitz, J.G., Yarbrow, J.W. and Weiss, A.J. Phase I study of subcutaneous 5-azacytidine in solid tumors. *Clin. Res.* 20:562, 1972.
- A6. Bellet, R.E., Mastrangelo, M.J., Bornstein, R.S., Engstrom, P.F. and Gerstley, B.J. A Phase I study of ICRP 159. *Clin. Res.* 20:884, 1972.
- A7. Mastrangelo, M.J., Kim, Y.H., Bornstein, R.S., Chee, D.O., Sulit, H.L. and Yarbrow, J.W. Clinico-pathological correlation of BCG-induced melanoma regression. *Proc. Am. Assoc. Cancer Res.* 14:65, 1973 (Abstract #257).
- A8. Mastrangelo, M.J., Bornstein, R.S., Sulit, H.L., Chee, D.O., Prehn, R.T. and Yarbrow, J.W. An immunological approach to the treatment of malignant melanoma. *J. Dent. Res.* 52:59, 1973 (Abstract #4).
- A9. Bodurtha, A.J., Laucius, J.F., Mastrangelo, M.J., Prehn, L.M. and Prehn, R.T. An immunological approach to the treatment of malignant melanoma. *Ann. R. Coll. Phys. Surg. Can.* 7:46, 1974 (Abstract #128).
- A10. Laucius, J.F., Bodurtha, A.J., Berkelhammer, J., Prehn, L.M., Prehn, R.T. and Mastrangelo, M.J. Intradermal injection of autochthonous irradiated tumor cells plus BCG in the treatment of metastatic malignant melanoma. *Proc. Am. Soc. Clin. Oncol.* 15:171, 1974 (Abstract #749).
- A11. Bodurtha, A.J., Chee, D.O., Laucius, J.F., Mastrangelo, M.J. and Prehn, R.T. Clinical and immunological significance of human melanoma cytotoxic antibody. *Proc. Am. Assoc. Cancer Res.* 15:63, 1974 (Abstract #251).
- A12. Creech, R., Catalano, R., Mastrangelo, M.J. and Engstrom, P.F. An effective low-dose three-drug regimen for metastatic breast cancer. *Proc. Am. Soc. Clin. Oncol.* 14:179, 1974 (Abstract #783).
- A13. Laucius, J.F., Bodurtha, A.J., Creech, R. and Mastrangel, M.J. Randomized prospective trial comparing BCNU plus vincristine (VCR) versus imidazole carboxamide (DTIC) in patients with metastatic malignant melanoma. *Proc. XI Int. Cancer Congr.* 3:540, 1974.

- A14. Berkelhammer, J., Mastrangelo, M.J. and Prehn, R.T. Sequential in vitro reactivity of lymphocytes from melanoma patients receiving immunotherapy compared with the reactivity of lymphocytes from healthy donors. Proc. Am. Assoc. Cancer Res. 16:94, 1975 (Abstract #375).
- A15. Engstrom, P.F., Catalano, R.B., Creech, R.H. and Mastrangelo, M.J. Chemoimmunotherapy of colorectal carcinoma with 5-FU + BCG. Proc. Am. Soc. Clin. Oncol. 16:234, 1975 (Abstract #1052).
- A16. Bellet, R.E., Mastrangelo, M.J., Creech, R.H. and Engstrom, P.F. Phase II study of ICRF-159 (NSC-129943) in human solid tumors. Proc. Am. Soc. Clin. Oncol. 16:224, 1975 (Abstract #1015).
- A17. Koons, L.S., Bellet, R.E. and Mastrangelo, M.J. Piperazinedione (NSC-135758) chemotherapy for advanced squamous cell carcinoma of the lung. Proc. Am. Soc. Clin. Oncol. 18:299, 1977 (Abstract #C-132).
- A18. Bellet, R.E., Mastrangelo, M.J., Berd, D. and Lustbader, E. Randomized prospective Phase III trial of methyl-CCNU (NSC-95441) alone versus methyl-CCNU + vincristine (NSC-62574) in the treatment of patients with metastatic malignant melanoma. Proc. Am. Soc. Clin. Oncol. 18:284, 1977 (Abstract #C-69).
- A19. Berd, D., Wilson, E., Bellet, R.E. and Mastrangelo, M.J. Methyl-CCNU adjuvant chemotherapy of malignant melanoma is not immunosuppressive. Am. Soc. Clin. Oncol. 19:349, 1978 (Abstract #C-171).
- A20. Mastrangelo, M.J., Bellet, R.E. and Berd, D. A randomized prospective trial comparing methyl-CCNU + vincristine with methyl-CCNU + vincristine + BCG + allogene tumor cells in patients with metastatic malignant melanoma. Am. Soc. Clin. Oncol. 19:319, 1978 (Abstract #C-52).
- A21. Bellet, R.E., Mastrangelo, M.J. and Berd, D. Phase II study of dibromodulcitol in patients with metastatic malignant melanoma. Proc. Am. Assoc. Cancer Res. 19:342, 1978 (Abstract #C-141).
- A22. Bellet, R.E., Mastrangelo, M.J., Berd, D. and Danna, V. Secondary animal tumor screening of chemotherapeutic agents utilizing a human melanoma-nude mouse panel. Proc. Am. Assoc. Cancer Res. 19:15, 1978 (Abstract #C-58).
- A23. Maguire, H., Berd, D., Biermann, W. and Mastrangelo, M.J. Increased immunological reactivity in patients treated with cyclophosphamide (Cy). Proc. Am. Assoc. Cancer Res. 21:203, 1980 (Abstract #814).
- A24. Maguire, H., Berd, D., Engstrom, P., Paul, A. and Mastrangelo, M.J. Cyclophosphamide (Cy) increases the acquisition of T-cell immunity in patients with melanoma and colorectal cancer. Proc. Am. Assoc. Cancer Res. 22:279, 1981 (Abstract #1106).
- A25. Berd, D., Parker, G., Shields, J., Morris, M. and Mastrangelo, M.J. Autologous mixed lymphocyte-tumor cell (AMLTC) response in primary choroidal melanoma. Proc. Am. Assoc. Cancer Res. 22:322, 1981 (Abstract #1279).

- A26. Berd, D., Danna, V., Maguire, H. and Mastrangelo, M.J. Selective impairment of suppressor T cell function after administration of cyclophosphamide (Cy). Proc. Am. Assoc. Cancer Res. 23:279, 1981 (Abstract #1104).
- A27. Mastrangelo, M.J. and Berd D. Current status of biological response modifiers therapy of human cancer. Proc. XIII Int. Cancer Congr. p. 9, 1982 (Abstract #40).
- A28. Berd, D., Maguire, H. and Mastrangelo, M. Augmentation of delayed type hypersensitivity (DTH) to tumor-associated antigens by treatment with autologous tumor cell vaccine preceded by cyclophosphamide (Cy). Proc. Am. Assoc. Cancer Res. 2:56, 1983 (Abstract #C-217).
- A29. Berd, D., Danna, V., Maguire, H.C. and Mastrangelo, M. Reduction of Con A-inducible suppressor T cell activity after administration of cyclophosphamide (Cy). Proc. Am. Assoc. Cancer Res. 24:207, 1983 (Abstract #816).
- A30. Berd, D., Danna, V., Maguire, H.C. and Mastrangelo, M. Cyclophosphamide (Cy) augments the development of delayed type hypersensitivity (DTH) to autologous human melanoma cells. Proc. Am. Soc. Clin. Oncol. 3:55, 1984 (Abstract #C-213).
- A31. Berd, D., Golden, M.H., Maguire, H.C. and Mastrangelo, M.J. Augmentation of cell-mediated and humoral immunity to a primary antigen by low dose cyclophosphamide (Cy) in patients with advanced cancer. Proc. Am. Assoc. Cancer Res. 25:273, 1984 (Abstract #939).
- A32. Berd, D., Golden, M.H., Maguire, H.C. and Mastrangelo, M.J. Comparison of effects of low dose versus high dose cyclophosphamide (Cy) on peripheral blood lymphocyte (PBL) composition and function. Proc. Am. Assoc. Cancer Res. 26:271, 1985 (Abstract #1067).
- A33. Berd, D., Golden M.H. and Mastrangelo, M.J. In vivo depletion of T-suppressor lymphocytes by administration of the monoclonal antibody anti-Leu 2a. Proc. Am. Assoc. Cancer Res. 27:311, 1986 (Abstract #1232).
- A34. Slovin, S.F., Lackman, R.D., Ferrone, S., McClay, E.F., Weiss, A.J. and Mastrangelo, M.J. Analysis of cellular immune response to sarcoma using cytotoxic T cell clones. Proc. Am. Assoc. Cancer Res. 27:360, 1986 (Abstract #1428).
- A35. McClay, E., Mastrangelo, M., Bellet, R. and Berd, D. An effective chemo/hormonal therapy regimen for the treatment of disseminated malignant melanoma. Proc. Am. Soc. Clin. Oncol. 6:208, 1987 (Abstract #821).
- A36. Berd, D., Golden, M.H., Clark, C., Hart, E. and Mastrangelo, M.J. Selective depletion of CD4+, 2H4+ suppressor-inducer T cells in patients treated with cyclophosphamide (Cy) and melanoma cell vaccine. Proc. Am. Assoc. Cancer Res. 28:376, 1987 (Abstract #1490).
- A37. Berd, D., Clark, C., Hart, E. and Mastrangelo, M.J. An unusual pattern of tumor regression in melanoma patients treated with cyclophosphamide (Cy) + autologous tumor cell vaccine. Proc. Am. Assoc. Cancer Res. 29:408, 1988 (Abstract #1626).
- A38. Slovin, S.F., Maguire, H.C., Jr., Lackman, R.D. and Mastrangelo, M.J. The role of "educator" tumor cells in restricting polyspecificity of killer cells. Proc. Am. Assoc. Cancer Res. 29:436, 1988 (Abstract #1735).

- A39. McClay, E., Sprandio, J., Mastrangelo, M., Bellet, R.E. and Berd, D. Importance of tamoxifen to a combination chemotherapy regimen for melanoma. Proc. Am. Soc. Clin. Oncol. 7:251, 1988 (Abstract #971).
- A40. Schultz, S., Kane, M., Roush, R., Miller, V., Berd, D., Goldman, L. and Mastrangelo, M.J. Inverse relationship of tumor thickness and time to recurrence in patients with clinical Stage I cutaneous melanoma. Proc. Am. Soc. Clin. Oncol. 8:285, 1989 (Abstract #1111).
- A41. Berd, D., Mastrangelo, M.J., Green, C., Clark, C. and Hart, E. Tumor inflammatory response induced by immunization with autologous melanoma cells conjugated to dinitrophenol (DNP). Proc. Am. Assoc. Cancer Res. 30:382, 1989 (Abstract #1515).
- A42. McClay, E.F., Mastrangelo, M.J., Bellet, R. and Berd D. An effective chemo/hormonal therapy regimen for the treatment of disseminated malignant melanoma. Update I. Proc. Am. Soc. Clin. Oncol. 8:282, 1989 (Abstract #1098).
- A43. Mastrangelo, M.J., Berd, D. and Maguire, H.C., Jr. Active immunotherapy of human melanoma with autologous vaccine and low dose cyclophosphamide (Cy). Proc. Second Int. Conf. Melanoma. Venice, Italy, 16-19 October 1989, p. 284.
- A44. Cardi, G., Bellet, R.E., Mastrangelo, M.J. and Berd, D. Selective growth of the CD56+CD16- subset of natural killer (NK) cells in melanoma tumor - infiltrating lymphocyte (TIL) cultures. Proc. Am. Assoc. Cancer Res. 31:271, 1990 (abstract #1605).
- A45. Slovin, S., Johnson, P., Patel, J., Cramer, J., Ferrone, S. and Mastrangelo, M.J. HLA-A2 in sarcomas: Isolation of immunoreactive HLA-A2 variant T cell subpopulations. Proc. Am. Assoc. Cancer Res. 31:267, 1990 (abstract #1580).
- A46. Berd, D., Maguire, H.C., Jr., Mastrangelo, M.J. and Murphy, G. Lymphocytic infiltration of melanoma metastases induced by immunization with dinitrophenol (DNP) - conjugated tumor cells. Proc. Am. Assoc. Cancer Res. 31:279, 1990 (abstract #1654).
- A47. Berd, D., Maguire, H.C., Jr., Clark, C., Green, C., Hart, E. and Mastrangelo, M.J. Treatment of metastatic melanoma with an autologous tumor cell vaccine: Clinical and immunological results in 64 patients. Proc. Am. Soc. Clin. Oncol. 9:278, 1990 (abstract #1079).
- A48. Berd, D., Clark, C., Green, C., Hart, E. and Mastrangelo, M.J. Activation markers on tumor-infiltrating T cells induced by dinitrophenyl (DNP)-conjugated autologous melanoma vaccine. Proc. Am. Assoc. Cancer Res. 32:256, 1991 (Abstract #1523).
- A49. Berd, D., McLaughlin, C.J., Hart, E., Wiebe, V.J., Mastrangelo, M.J., Bellet, R.E., and DeGregorio, M.W. Short course high-dose tamoxifen (TAM) with cytotoxic chemotherapy for metastatic melanoma. Proc. Am. Soc. Clin. Oncol. 10:291, 1991 (Abstract #1016).
- A50. Kane, M., Laucius, J.F., Bellet, R., Berd, D., Mastrangelo, M., Rose, L. and Rabin witz, M. Phase I/II trial of etoposide (E) and cef p raz ne (C) in the treatment of refractory ne plasms. Proc. Am. Soc. Clin. Oncol. 10:111. 1991 (Abstract #315).

- A51. Somasundaram, R., Benden, A., Zaloudik, J., Sperlagh, M., Hart, E., Marks, G., Mastrangelo, M. and Herlyn, D.: Antigen-specific humoral and cellular immune response in colorectal carcinoma patients immunized with anti-idiotypic antibody GA733. Proc Am Assoc Cancer Res 33:331, 1992 (Abstract #1977).
- A52. Maguire, H.C., Jr., McCue, P., Berd, D., Lattime, E.C. and Mastrangelo, M.J.: Assessment of the effects of R-24 antibody on selected parameters of immunity. Annual Meeting Society for Biological Therapy, Williamsburg, VA 10/29-11/1/92. J. Immunother. 13:65, 1993.
- A53. Mastrangelo, M.J.: Review of chemotherapy and immunotherapy for melanoma. Cancer Investigation 11 (Suppl. 1): 52-54, 1993 (Abstract #43).
- A54. Mastrangelo, M.J., Nathan, F., Maguire, H.C., Jr., and Berd, D.: Trials with combination chemotherapy and with active specific immunotherapy. Melanoma Res. 3 (Suppl. 1):33, 1993 (Abstract #114).
- A55. Lee, S.S., Eisenlohr, L.C., McCue, P.A., Mastrangelo, M.J. and Lattime, E.C. Intravesical gene therapy: Vaccinia virus recombinants transfect murine bladder tumors and urothelium. Proc Am Assoc Cancer Res 34:337, 1993 (Abstract #2009).
- A56. Berd, D., Maguire, H.C., Jr., Hart, E. and Mastrangelo, M.J.: Post-surgical adjuvant therapy of melanoma with a dinitrophenyl (DNP) - conjugated vaccine: Prolongation of disease-free and total survival. Proc Am Soc Clin Oncol 12:287, 1993 (Abstract #1319).
- A57. Mastrangelo, M.J., Hart, E. and Berd, D.: Dinitrophenyl (DNP)-conjugated autologous melanoma vaccine and PEG-IL2. Ann Hematol 67 (Suppl II): A170, 1993 (Abstract #105).
- A58. Lattime, E.C., Mastrangelo, M.J. and Berd, D.: Human metastatic melanoma lesions and cell lines express mRNA for IL-10. Proc Am Assoc Cancer Res (submitted).
- A59. Lattime, E.C., Maguire, H.C., Jr., McCue, P.A., Eisenlohr, L.C., Berd, D., Lee, S.S. and Mastrangelo, M.J.: Infection of human melanoma cells by intratumoral vaccinia. J. Investigative Dermatol (Abstract) (Submitted 12/93).
- A60. Lattime, E.C., Maguire, H.C., Jr., McCue, P.A., Eisenlohr, L.C., Berd, D., Lee, S.S. and Mastrangelo, M.J.: Gene therapy using vaccinia vectors: Repeated intratumoral infections in the presence of anti-vaccinia immunity. Proc. Am. Soc. Clin. Oncol. (submitted 12/93).
- A61. Lee, S.S., Eisenlohr, L.C., McCue, P.A., Mastrangelo, M.J. and Lattime, E.C. Vaccinia virus vector mediated cytokine gene transfer for in vivo tumor immunotherapy. Proc Am Assoc Cancer Res (Submitted 11/93).
- A62. Nathan, F.E., Sato, T., Hart, E., Berd, D. and Mastrangelo, M.J.: Response to combination chemotherapy of liver metastases from choroidal melanoma compared with cutaneous melanoma. Proc Am Soc Clin Oncol (Submitted 12/93).
- A63. Berd, D., Hart, E., Nathan, F., Fishbein, G. and Mastrangelo, M.J.: Chemotherapy of melanoma with BCNU-Dacarbazine-cisplatin + tamoxifen: A nine year perspective. Proc Am Soc Clin Oncol (Submitted 12/93).

- A64. Berd, D., Maguire, H.C., Hart, E., and Mastrangelo, M.J.: Immunotherapy of melanoma with a dinitrophenyl (DNP)-conjugated vaccine: Follow-up of a post-surgical adjuvant study. Proc Am Soc Clin Oncol. (Submitted 12/93).



CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on November 4, 1996.

  
Nancy J. Robins

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Lynn E. Spitler et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

**DECLARATION OF ROBERT OLDHAM, MD  
PURSUANT TO 37 C.F.R § 1.132**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, Robert Oldham, MD, declare as follows:

1. I am the Director of the Biological Therapy Institute in Franklin, Tennessee. A copy of my *Curriculum Vitae* is attached hereto as Exhibit A. I am a consultant to Jenner Technologies, the assignee of the present application.

2. I have reviewed the Declaration Under 37 C.F.R. 1.132 prepared by Dr. Lynn E. Spitler describing the results of a clinical study directed to the use of prostate specific antigen (PSA) as an active ingredient in an antiprostata cancer vaccine. I am also familiar with the study itself, and with the results that were obtained.

3. The purpose of the study was to obtain evidence that the vaccines would raise a sufficient cellular immune response to have a beneficial effect with respect to prostate tumors. Such a result could be shown directly by measuring cytotoxic lymphocyte (CTL) generation; however, I am aware that this was not possible in these studies because the assay was not satisfactory because of the lack of an appropriate target cell for the assay.


4. The responses measured are understood in the art to be satisfactory substitutes for measuring CTLs. Thus, the proliferation of lymphocytes from two of the patients in response to contact with PSA or in response to peptides representing putative PSA epitopes is indicative of an appropriate cellular immune response. The ability of PSA or PSA derived peptides to stimulate cytokine production -- i.e., gamma interferon and IL-4 production -- from lymphocytes in these patients also indicates that the cellular response is obtained specifically with respect to PSA. The observation of the development of a positive skin test response to PSA in one patient is also consistent with these observations showing the development of cell-mediated immunity in the patients.

5. In my opinion, the results obtained in this clinical study provide evidence that the vaccines are likely to be effective in exerting a beneficial effect on patients with prostate tumors or at risk for prostate tumors.

6. The efficacy shown for the vaccine tested in the foregoing clinical studies further provides evidence that analogous vaccines based on host tissue antigen, such as prostate specific membrane antigen (PSMA) and prostate acid phosphatase (PAP) would behave in a similar manner. It is also well known that if the entire antigen is effective as a vaccine, portions of the antigen will be effective as well, especially if manipulated by art-known methods to enhance their immunogenicity, such as by coupling them to carrier.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

October 10, 1996

  
Robert Oldham, MD

Revised: 10/1/95

**CURRICULUM VITA**

**Name:** Robert K. Oldham, M.D.

**Permanent Address** 357 Riverside Drive  
Franklin, Tennessee 37064  
615/791-4073

**Social Security #:** 500-40-8139

**Date/Place of Birth:** 9/16/41; Pocatello, Idaho

**Marital Status:** Single (5 sons)

**College:** University of Missouri, Columbia, MO  
Chemical Engineering  
Pre-Medical Sciences

**Medical School:** 1968, Medical Degree  
University of Missouri  
Columbia, Missouri

**Graduate Training:** 1970-1971 Immunology  
NIH Graduate Program

**Professional Employment**

1991 - Present Chairman of the Board and President  
Cancer Therapeutics Inc.  
357 Riverside Drive  
Franklin, Tennessee 37064

1992 - Present Chairman of the Board and President  
American Patient Services  
357 Riverside Drive  
Franklin, Tennessee 37064

1986 - Present Chairman of the Board  
Media America  
357 Riverside Drive  
Franklin, Tennessee 37064

**Consultant:**

- 1984 - 1986 Wellcome Biotechnology Ltd.  
Kent, England
- 1989 - Present Amersham International  
Buckinghamshire, England
- 1991 - Present Cancer Treatment Centers of America  
Zion, Illinois
- 1994 - Present Applied Immune Sciences  
Santa Clara, California 95054

**Editorial Boards:**

- 1992 - Present Cancer Biotherapy, Founder, (now Cancer Biotherapy & Radio-pharmaceuticals) Editor-in-Chief
- 1990 - Present In Vivo, Editorial Board  
Natural Immunity and Cell Growth Regulation, Editorial Board
- 1988 - 1992 Molecular Biotherapy, Founder and Editor-in-Chief
- 1982 - 1989 Journal of Biological Response Modifiers (now Journal of Immunotherapy) Founder and Editor-in-Chief
- 1982 - 1985 Cancer Immunology and Immunotherapy, Editorial Board

**Major Research Interests:**

- Cancer Biotherapy
- Biologicals and Biological Response Modifiers
- Investigative Trials in Clinical Oncology
- Cellular Therapy of Cancer
- Monoclonal Antibodies & Immunoconjugates in Cancer Treatment
- In Vitro Assays in Tumor Immunology
- Privatization of Cancer Research

**Professional Employment (Continued)**

1984 - Present	Founder and Director Biological Therapy Institute 357 Riverside Drive Franklin, Tennessee 37064
1984 - Present	Clinical Professor of Medicine Hematology/Oncology University of Missouri Columbia, Missouri 65212
1984 - 1990	Founder, Scientific Director and Chairman of the Board Biotherapeutics Inc. (Now Response Technologies, Inc.) Technologies, Inc.) Memphis, Tennessee 38117
1984 - 1986	Founder and Consultant Biomedical Research Center University of British Columbia Vancouver, British Columbia V6T 2B5
1980 - 1984	Founding Director, Biological Response Modifiers Program Associate Director, Division of Cancer Treatment National Cancer Institute Frederick, Maryland 21701
1975 - 1980	Founder and Director, Div. of Oncology Associate Professor of Medicine Associate Director, Cancer Center Vanderbilt University Medical Center Nashville, Tennessee 37232

**Professional Employment Continued:**

1974 - 1975	Senior Investigator Laboratory of Immunodiagnosis National Cancer Institute Bethesda, Maryland 20205
1973 - 1974	Senior Investigator Cellular and Tumor Immunology Section Laboratory of Cell Biology National Cancer Institute Bethesda, Maryland 20205
1972 - 1973	Research Associate with Professor G. Mathe ICIG - Hospital Paul Brousse Villejuif, France
1971 - 1972	Clinical Associate, Cellular and Tumor Immunology Section Laboratory of Cell Biology National Cancer Institute Bethesda, Maryland 20205
1970 - 1971	Clinical Associate, Radiation Branch National Cancer Institute Bethesda, Maryland 20205
1968 - 1970	Internal Medicine Intern and Resident Vanderbilt University Hospital Nashville, Tennessee 37205

**Clinical Group Appointments:**

1976 - 1980	Principal Investigator - Vanderbilt Southeastern Oncology Study Group (NCI)
1978 - 1980	Principal Investigator - Vanderbilt Lung Cancer Study Group (NCI)
1986 - 1990	Founder and Group Chairman - National Biotherapy Study Group
1986-Present	Principal Investigator - Biological Therapy Institute - National Biotherapy Study Group

**Fellowship Experience:**

1970 - 1972	Medical Oncology Fellowship, National Cancer Institute - Bethesda, Maryland
1967	PHS Cancer Clinical Fellowship, Lemuel Shattuck Hospital-Jamaica Plain - Boston, Massachusetts
1967	PHS Cancer Clinical Fellowship, Ellis Fischel Cancer Hospital - Columbia, Missouri
1965	PHS Student Research Fellowship, University of Missouri Medical School - Columbia, Missouri

**Honors, Awards and Societies:**

1967	Alpha Omega Alpha
1970	Diplomat, National Board of Medical Examiners
1975	Diplomat, American Board of Internal Medicine
1975	Diplomat, Medical Oncology, American Board of Internal Medicine
1976	American Association of Immunologists
1976	International Society for Experimental Hematology
1976	Williamson County Medical Society
1977	Southern Medical Association
1977	American Federation for Clinical Research
1977	American Society for Clinical Oncology
1978	American College of Physicians, Fellow
1979	Southern Society for Clinical Investigation
1982	American Osler Society
1984	Society for Biological Therapy, Founder President - May 1984 to November 1986
1984	Reticuloendothelial Society
1986	National Biotherapy Study Group, Founder Group Chairman - 1986 to 1990
1990	Association of Community Cancer Centers
1994	International Cytokine Society
1994	The American Association of Bioethics

**Licensure:**

Tennessee, Maryland and Missouri



**Major Accomplishments** (Curriculum Vitae #)

Discovery of Natural Killer Cells

(#8, 9, 27, 28, 31, 46, 47, 56, 90, 92, 121, 157 323)

Development of improved therapy for small cell lung cancer  
(#66, 69, 78, 80, 85, 95, 103, 105, 108, 124, 177)

Development of improved therapy for ovarian cancer  
(#99, 102, 125)

Development of improved methods in detecting and treating extragonadal germ cell tumors and other poorly differentiated tumors  
(#22, 87, 93, 122, 123, 227, 228; 5-Book)

Use of cryopreserved cells for standardized immunological testing  
(#37, 38, 40, 41, 45, 49, 52, 120)

Cancer Biotherapy: first to use the term to describe a fourth modality of cancer treatment  
(#109, 114, 119, 128, 129, 134, 137, 139, 141, 146, 151 153, 154, 161, 169, 173, 174, 175, 176, 179, 181, 183, 184, 188, 193, 197, 204, 206, 212, 225, 240, 249, 250, 184, 188, 193, 197, 204, 206, 212, 225, 240, 249, 250, 252, 260, 261, 262, 264, 265, 266, 267, 301, 302, 314, 319, 322, 324, 325, 332, 333, 338, 339, 340, 343, 344, 346, 349, 356, 357, 358, 360, 361, 362, 363) 10 Books)

Monoclonal antibodies and immunoconjugates in cancer treatment: Did groundbreaking experiments with toxin, drug and isotope conjugates  
(#125, 126, 140, 143, 144, 145, 150, 152, 155, 156, 159, 160, 165, 180, 181, 182, 185, 187, 195, 196, 198, 199, 206, 218, 223, 224, 235, 238, 241, 244, 246, 247, 248, 251, 253, 254, 263, 278, 279, 285, 286, 287, 288, 291, 306, 307, 310, 311, 318, 325, 327, 342, 347)

Cytokine and Cellular Therapy: Conducted some of initial clinical experiments with IL-2 in LAK and T cell therapy  
(#249, 255, 259, 281, 290, 294, 295, 298, 301, 302, 309, 311, 314, 322, 324, 332, 333, 349, 356, 357, 358, 361, 362)

Individualized Cancer Treatment: Conducted specific laboratory and clinical experiments demonstrating that each cancer in each patient has unique characteristics which may require unique, individualized treatment  
(#267, 279, 285- 288, 291, 306, 307, 310, 318, 325, 327, 342, 347)

Privatization of Cancer Research: Brought attention to clinical research opportunities in the private sector. Published widely on problems of access to new technologies and the funding of clinical research  
(#134, 205, 206, 207, 231, 236, 245, 256, 257, 268, 269, 282, 284, 289, 293, 308, 312, 316, 317, 319, 321, 325, 326, 330, 331, 334, 335, 350, 352, 353, 354, 355, 358, 359, 364, 11-Books)

### Publications

Over 330 papers published in the medical/scientific literature

Thousands of abstracts, posters and presentations at various meetings on cancer research and treatment

Editor of Principles of Cancer Biotherapy, the first comprehensive textbook on the fourth modality of cancer treatment (Now in Third Edition).

Founding Editor of Cancer Biotherapy, (now Cancer Biotherapy & Radiopharmaceuticals), Molecular Biotherapy and the Journal of Biological Response Modifiers (now Journal of Immunotherapy).

Author or editor of thirteen books on cancer research and treatment

## BIBLIOGRAPHY

### Published Papers

- 1 Oldham, R K Terminal cancer - A patient oriented approach J Tenn Med Assoc , 63 206, 1970.
- 2 Oldham, R K Aseptic meningitis following the intrathecal injection of RISA Radiology, 97:317, 1970
- 3 Oldham, R K Eosinophilic Granuloma South. Med J , 64 978, 1971
- 4 Oldham, R K and Pomeroy, T C Vincristine induced inappropriate ADH syndrome. South Med.J , 65 1010-1012, 1972
- 5 Oldham, R K and Pomeroy, T C Treatment of Ewing's sarcoma with Adriamycin Cancer Chemotherapy Reports, 56 635-639, 1972
- 6 Oldham, R K and Pomeroy, T C Extramedullary plasmacytomas following successful radiotherapy of Hodgkin's disease Clinical and immunological aspects American J. Med., 54 761-767, 1973.
- 7 Oldham, R K , Larson, S M., and Givelber, H.M , Chretien, P B , and Johnson, R E. A preliminary study of <sup>51</sup>Cr-labeled platelets for evaluation of splenic sequestration in chronic lymphocytic leukemia J Nuclear Med , 37,219-222, 1973
- 8 Oldham, R.K., Siwarski, D , McCoy, J.L , Plata, E J , and Herberman, R.B . Evaluation of a cell-mediated cytotoxicity assay utilizing <sup>125</sup>Iododeoxyuridine labeled tissue culture targets Nat Cancer Inst Monogr., 37:49-58, 1973
- 9 Oldham, R K., Herberman, R.B Evaluation of cell-mediated cytotoxic reactivity against tumor associate antigen utilizing <sup>125</sup>Iododeoxyuridine labeled target cells J Immunol., 111 1862-1971, 1973
- 10 Lemevel, B P , Oldham, R K., Wells, S A , and Herberman, R B. An evaluation of <sup>125</sup>Iododeoxyuridine as a cellular label for in vitro assays - Kinetics of incorporation and toxicity J Nat Cancer Inst , 51 1511-1558, 1973
- 11 Oldham, R K and Simmler, M C The use of cryopreserved lymphocytes and lymphoblasts in <sup>51</sup>Cr lymphocyte cytotoxicity In Weiner, R S , Oldham, R K., and Schwarzenberg, L.(Eds.) The Cryopreservation of Normal and Neoplastic Cells, Inserm, Institute National de la Sante et de la Recherche Medicale Paris, pp 161 169, 1973
- 12 Oldham, R K and Simmler, M C Possible role of lymphocyte cytotoxicity in bone marrow grafting Trans. Proceed., 6 417, 1974
- 13 Jasmin, C , Bricout, F., Huraux, J M , Weiner, R., Oldham, R K., and Mathe, G.: A study of viral infections in patients treated with a combination of 6 meraptopurine-methotrexate: Preliminary results. In Mathe, G., and Oldham, R K (Eds.) Recent Results in Cancer Research Complications of Cancer Chemotherapy, Vol 49 New York, Springer-Verlag. pp. 29-33 1974

14. Mathe, G. and Oldham, R.K. Introduction. In Mathe, G. and Oldham, R.K. (Eds.): Complications of Cancer Chemotherapy. New York, Springer-Verlag, pp. 1-2, 1974.
15. Beard, J., Weiner, R.S., Oldham, R.K., and Mathe, G.: Immune responsiveness in acute lymphocytic leukemia patients under chemotherapy and immunotherapy. A preliminary report. In Mathe, G. and Oldham, R.K. (Eds.): Recent Results in Cancer Research: Complications of Cancer Chemotherapy. New York, Springer-Verlag, pp. 56-60, 1974.
16. Belpomme, D., Carde, P., Oldham, R.K., Mathe, G., Jacquillat, N., Chelloul, N., Weil, M., Auclerc, C., Weisgerber, G., Tanzer, T., and Bernard, J.: Malignancies possibly secondary to anticancer therapy. In Mathe, G. and Oldham, R.K. (Eds.): Recent Results in Cancer Research: Complications of Cancer Chemotherapy. New York, Springer-Verlag, pp. 115-123, 1974.
17. Mathe, G., Schwarzenberg, L., Pouillart, P., Wiener, R., Oldham, R.K., Jasmin, C., Hayat, M., Schneider, M., Amiel, J.L., Ceoara, B., and Steresco-Musset, M.: Essai de Traitement de divers hematiosarcomas par le 4-demethyl-epipodophyllotoxine beta.D. thenylidene glucoside (VM 26 ou EPT). La Nouvelle Presse Med., 3:337-451, 1974.
18. Mathe, G., Schwarzenberg, L., Pouillart, P., Wiener, R., Oldham, R.K., Jasmin, C., Hayat, M., Schneider, M., Amiel, J.L., Ceoara, B., and Steresco-Musset, M.: Leucemies aigues et hematiosarcomas divers: Essai de traitement par un second derive de la podophyllotoxine (le 4-Demethylepipodophyllotoxine Beta.D. ethylidene glucoside VP16-213 ou EPE). La Nouvelle Presse Med., 3:521-524, 1974.
19. Mathe, G., Schwarzenberg, L., Pouillart, P., Oldham, R.K., Wiener, R., Jasmine, C., Hayat, M., Schneider, M., Amiel, J.L., de Vassal, F.: Two epipodophyllotoxin derivatives, VP 26 and VP16-213 in the treatment of leukemias and hemtiosarcomas. In Bucalossi, P., Veronesi, U., Bonadonna, G., and Errianuelli, H. (Eds.): I Linfomi Maligni. Milano, Casa Editrice Ambrosiana, pp. 303-309, 1974.
20. Mathe, G., Schwarzenberg, L., and Oldham, R.K.: le controle et le traitement de l'insuffisance medullaire qui peut compliquer les lymphomes et/ou leurs traitements. In Bucalossi, P., Veronesi, U., Bonadonna, G., and Emanuele, I. (Eds.): I Linfomi Maligni. Milano, Casa Editrice Ambrosiana, pp. 359-365, 1974.
21. Herberman, R.B., Char, D., Oldham, R.K., Levine, P., Leventhal, B.G., and McCoy, J.L.: Cell-mediated immunity in human acute leukemia. In Ito, Y., and Dutcher, R.M. (Eds.): Comparative Leukemia Research. University of Tokyo/Karger, pp. 585-592, 1974.
22. Mathe, G., Schwarzenberg, L., Pouillart, P., Oldham, R.K., Weiner, R., Kasom, C., Hayat, M., Sachneider, M., Amiel, J.L., and de Vassa, F.: Two epipodophyllotoxin derivatives, VP 26 and VP16-213, in the treatment of leukemias and hematiosarcomas. Cancer, 34:985-992, 1974.
23. Herberman, R.B., Hollinshead, A., Char, D., Oldham, R.K., McCoy, J.L., and Cohen, M.: In vivo and in vitro studies of cell-mediated immune response to antigens associated with malignant melanoma. In International Symposium on Immuno-logical Reactions to Melanoma Antigens. Behring Institute Research Communications, Behringwerke AG. West Germany, Marburg Publisher, No. 56, pp. 131-138, 1974.

24. Herberman, R.B., Char, D.H., Oldham, R.K., and Leventhal, B.G. The prognostic value of studies of specific cell-mediated immunity in acute leukemia. In T.M. Fleidner and S. Perry (Eds.): *Advances in the Biosciences* 14. Workshop on Prognostic Factors in Human Acute Leukemia. Branschweig, West Germany, Friedr. Viewegh and Sohn, pp. 431-440, 1975.
25. Pouillart, P., Weiner, R., Scharzenberg, L., Misset, J.L., Oldham, R.K., Amiel, J.L., Mathe, G. Combination chemotherapy based on a model of cell recruitment by partial synchronization. *Med. and Ped. Oncology*, 1:123-134, 1975.
26. McCoy, J.L., Jerome, L.F., Dean, J.H., Perlman, E., Oldham, R.K., Char, D.H., Cohen, M.H., Felix, E.L., and Herberman, R.B.: Inhibition of leukocyte migration by tumor-associated antigens in soluble extract of human malignant melanoma. *J. Natl. Cancer Inst.*, 55:19-23, 1975.
27. Herberman, R.B. and Oldham, R.K.: Problems associated with study of cell-mediated immunity to human tumors by microcytotoxicity assays. *J. Natl. Cancer Inst.*, 55:749-753, 1975.
28. Oldham, R.K., Djeu, J.Y., Cannon, G.B., Siwarski, D., Herberman, R.E.: Cellular microcytotoxicity in human tumor systems: Analysis of results. *J. Natl. Cancer Inst.*, 55:1305-1318, 1975.
29. Catalana, W.L., Oldham, R.K., Djeu, J.Y., Herberman, R.B., and Cannon, G.B.: Specificity of in vitro cellular cytotoxicity against transitional cell carcinoma cell line T-24. *Surgical Forum*, 26:122-124, 1975.
30. Law, J.P., Koch, F.J., Cannon, G.B., Herberman, R.B., and Oldham, R.K.: Acute myelomonocytic leukemia associated with paraproteinemia. *Cancer*, 37:1358-1364, 1976.
31. Oldham, R.K., Weiner, R.S., Mathe, G., Breard, J., Simmler, M.C., Carde, P., and Herberman, R.B.: Cell-mediated immune responsiveness of patients with acute lymphocytic leukemia in remission. *Int. J. Cancer*, 17:326-337, 1976.
32. McCoy, J.L., Dean, J.H., Cannon, G.B., Maurer, B.A., Oldham, R.K., and Herberman, R.B.: Detection of cell-mediated immunity against tumor associated antigens of human breast carcinoma by migration inhibition and lymphocyte stimulation assays. In J. Wybran and M. Staquet (Eds.): *Clinical Tumor Immunology*. Oxford, England, Pergamon Press, pp. 77-86, 1976.
33. Campbell, D.A., Oldham, R.K., Ortaldo, J.R., Nunn, M.E., Holden, H.T., Herberman, R.B.: Effect of X-irradiation on tumor associated antigens. In: Neibings, H.E. (Eds.): *Detection and Presentation of Cancer*. New York, Marcel Dekker, Inc., pp.447-465, 1978.
34. Herberman, R.B., Campbell, D.A., Jr., Oldham, R.K., Bonnard, G.D., Ting, C.C., Holden, H.T., Glaser, M., Djeu, J., and Oehler, R.: Immunogenicity of tumor antigens. *Ann. N.Y. Acad. of Sci.*, 276:26-39, 1976.
35. Oldham, R.K. and Herberman, R.B.: Determination of cell-mediated cytotoxicity by the 125Iododeoxyuridine microcytotoxicity assays. Bloom, B.R., and David, J.R. (Eds.): In: *In Vitro Immunity*. Academic Press, New York, pp. 461-470, 1976.

36. Herberman, R.B., Oldham, R.K., Connor, R.J.: Statistical analysis and selection of baseline control in cytotoxicity assays. Bloom, B.R. and David, J.R. (Eds.): In Vitro Methods in Cell Mediated and Tumor Immunity. Academic Press, New York, 481-488, 1976.
37. Holden, H.T., Oldham, R.K., Ortaldo, J.R., Herberman, R.B.: Cryopreservation of the functional reactivity of normal and immune leukocytes and of tumor cells. Bloom, B.R. and David, J.R. (Eds.): In Vitro Methods in Cell Mediated and Tumor Immunity. Academic Press, New York, pp. 723-729, 1976.
38. Ortaldo, J.R., Oldham, R.K., Holden, H.T., Herberman, R.B.: Immune response to Gross virus induced lymphoma: Cryopreservation of functional activity of rat lymphocytes and tumor cells. *Cell. Immunol.* 25:60-73, 1976.
39. McCoy, J.L., Jerome, L.F., Anderson, C., Cannon, G.B., Alford, T.C., Connor, R.J., Oldham, R.K., and Herberman, R.B.: Leukocyte migration inhibition by soluble extracts of MCF-7 tissue culture cell line derived from breast carcinoma. *J. Natl. Cancer Inst.* 57:1045-1049, 1976.
40. Oldham, R.K., Dean, J., Cannon, G.B., Graw, R., Dunston, G., Applebaum, F., McCoy, J., Djeu, J. and Herberman, R.B.: Cryopreservation of human lymphocyte function as measured by in vitro assays. *Int. J. Cancer*, 18:145-155, 1976.
41. Oldham, R.K., Weese, J.L., Herberman, R.B., Perlman, E., Mills, M., Heim, W., Blom, J., Green, D., Reid, J., Bellinger, S., Law, I., McCoy, J.L., Dean, J.H., Cannon, G.B., Djeu, J.: Immunological monitoring and immunotherapy in carcinoma of the lung. *Int. J. Cancer*, 18:739-749, 1976.
42. Campbell, D.A., Manders, E.K., Oehler, J.R., Bonnard, G.D., Oldham, R.K., Herberman, R.B.: Inhibition of in vitro lymphoproliferative response by in vivo passaged rat 13762 mammary adenocarcinoma cells. I: Characteristics of inhibition and evidence for an infectious agent. *Cell. Immunol.*, 33:363-377, 1977.
43. Campbell, D.A., Jr., Staal, S.P., Manders, E.K., Bonnard, G.D., Oldham, R.K., Salzman, L.A., Herberman, R.B.: Inhibition of in vitro lymphoproliferative responses by in vivo passaged rat 13762 mammary adenocarcinoma cells. II: Evidence that Kilham rat virus is responsible for the inhibitory effect. *Cell. Immunol.* 33:378-391, 1977.
44. Djeu, J., Payne, S., Alford, C., Heim, W., Pomeroy, T., Cohen, M., Oldham, R.K., Herberman, R.B.: Detection of decreased proportion of lymphocytes forming rosettes with sheep erythrocytes at 29 C in the blood of cancer patients: Analysis of factors affecting the assay. *Clinical Immunol. and Immunopath.*, 8:405-412, 1977.
45. Holden, H.T., Oldham, R.K., Ortado, J.R., and Herberman, R.B.: Standardization of the <sup>51</sup>Cr release cell-mediated cytotoxicity assay: Cryopreservation of mouse effector and target cells. *J. Natl. Cancer Inst.* 58:611-622, 1977.
46. Oldham, R.K., Ortaldo, J., Herberman, R.B.: Natural cytotoxic reactivity of rat lymphocytes as measured by the (125I) iododeoxyuridine and tritiated proline microcytotoxicity assays in the Gross virus induced lymphoma system. *Cancer Res* 32 4457-4463, 1977.

86. Lujek, M.A., Fer, M.F., Oldham, R.K., Greco, F.A., Roger, L.W.: Cushing's syndrome with metastatic nonkeratinizing carcinoma of the cervix. Report of a case and literature review. *Am. J. Med.*, 69:140-144, 1980.
87. Williams, S.D., Einhorn, L.H., Greco, F.A., Oldham, R.K., Fletcher, R.: VP-16-213. Salvage therapy for refractory germinal neoplasms. *Cancer*, 46:2154-2158, 1980.
88. Reid, J.W., Perlin, E., Oldham, R.K., Weese, J.L., Heim, W., Mills, M., Millar, C., Blom, J., Green, D., Ballinger, S., Cannon, G.B., Law, I., Connor, R., Herberman, R.B.: Immunotherapy of carcinoma of the lung with intradermal BCG and allogenic tumor cells: a clinical trial. In Terry, W. (Ed.): *Immunotherapy of Cancer: Present Status of Trials in man*. New York, Raven Press, 1980.
89. Knost, J.A., Reynolds, V., Greco, F.A., Oldham, R.K.: Adjuvant chemoimmunotherapy stage I/II malignant melanoma. *J. Surg. Oncol.*, 19:165-170, 1982.
90. Forbes, J.T., Niblack, G.D., Fuchs, R., Richie, R.E., Johnson, H.K., and Oldham, R.K.: Human natural cell-mediated cytotoxicity I. Levels in peripheral blood, cord blood, and thoracic duct lymphocytes. *Cancer Immunol. and Immunother.*, 11:139-146, 1981.
91. Forbes, J.T., Greco, F.A., Oldham, R.K.: Human natural cell-mediated cytotoxicity II. Levels in neoplastic disease. *Cancer Immunol. and Immunother.*, 11:139-146, 1981.
92. Forbes, J.T., Oldham, R.K.: Kinetic analysis of specificity in human natural cell-mediated cytotoxicity. In Herberman, R.B. (Ed.): *Natural Cell-Mediated Immunity Against Tumors*. New York, Academic Press, 819-834, 1980.
93. Richardson, R., Schoumacher, R., Oldham, R.K., Fer, M., Hande, R., Forbes, J.T., and Greco, F.A.: The unrecognized extragonadal germ cell cancer syndrome. *Ann. Int. Med.*, 94:181-186, 1981.
94. Greco, F.A., Hande, R.K., Richardson, R.L., and Oldham, R.K.: High dose methotrexate in combination chemotherapy in small cell lung cancer. In Mathe and Muggia, (Eds.): *Recent Results in Cancer Research*. New York, Plenum Press, 74:50-55, 1980.
95. Greco, F.A., Oldham, R.K.: Combined therapy in limited stage small cell bronchial carcinoma. In Whitehouse, M. and Williams, C. (Eds.): *Recent Advances in Clinical Oncology*. London, England, Churchill Livingstone, 325-339, 1982.
96. Gail, M.H., Oldham, R.K., Holmes, E.C., Wright, P.W., McGuire, W.B., Mountain, C.F., Lukeman, J.M., Feld, R., Hill, L.D., Eagan, R.T., Pearson, F.G., and Lung Cancer Study Group: Early of intrathoracic BCG therapy in patients with non-stage I lung cancer: data from a randomized double-blind controlled trial by the lung cancer study group. *Cancer Immunol. and Immunother.* 10:129-137, 1981.
97. Knost, J.A., Oldham, R.K., Hande, K.R., Rhamy, R.K., Greco, F.A.: The combination of vinblastine and bleomycin in metastatic renal cell carcinoma. *Cancer Treat. Rep.*, 65:349-350, 1981.
98. Knost, J.A., Greco, F.A., Hande, K.R., Richardson, R.L., Fer, M.F., Oldham, R.K.: Cyclophosphamide, Doxorubicin, and Cis. Platinum in the treatment of Small Cell Lung Cancer.

99. Oldham, R.K., Julian, C.G., Burnett, L.S., Richardson, R.L., Hande, K.R., Greco, F.A., Williams, C. (Eds): Recent Advances in Clinical Oncology. London, England, Churchill Livingstone, 165-179, 1982.
100. Dooley, W.C., Oldham, R.K.: Possible association between radiation exposure and chromosome changes. *Lancet*, 2(8185):98, 1980.
101. Fer, M.F., Greco, F.A., Haile, K.L., Rosenblatt, P.A., Johnson, R.L., Glick, A.D., Oldham, R.K.: Unusual survival after pulmonary artery sarcoma. *South Med. J.*, 74:624-627, 1981.
102. Greco, F.A., Julian, C.G., Richardson, R.L., Burnett, L., Hande, K.R., Oldham, R.K.: Advanced ovarian cancer: brief intensive combination chemotherapy and second look operation. *Obstet. Gynecol.*, 58:199-205, 1981.
103. Greco, F.A., Oldham, R.K.: Clinical management of patients with small cell lung cancer. In Greco, F.A., Oldham, R.K., Bunn, P.A. (Eds.): *Small Cell Lung Cancer*. New York, Grune and Stratton, 353-379, 1981.
104. Greco, F.A., Hainsworth, J.D., Sismani, A., Richardson, R.L., Hande, K., Oldham, R.K.: Hormone production and paraneoplastic syndromes. In Greco, F.A., Oldham, R.K., Bunn, P.A. (Eds.): *Small Cell Lung Cancer*. New York, Grune and Stratton, 177-223, 1981.
105. Forbes, J.T., Greco, F.A., Oldham, R.K.: Immunobiology of small cell lung cancer. In Greco, F.A., Oldham, R.K., Bunn, P.A. (Eds.): *Small Cell Lung Cancer*. New York, Grune and Stratton, 327-353, 1981.
106. Oldham, R.K.: Biological response modifier therapy. An overview, *The Cancer Bulletin*, 33: 244-250, 1981.
107. Lyles, J.N., Burish, T.G., Krozely, M.G., and Oldham, R.K.: Efficacy of relaxation training and guided imagery in reducing the aversiveness of cancer chemotherapy. *J. Con. Psychol.*, 50: 509-524, 1982.
108. Hande, K.R., Oldham, R.K., Fer, M.F., Richardson, R.L., and Greco, F.A.: Randomized study of high-dose vs. low-dose methotrexate in the treatment of extensive stage small cell lung cancer. *Am. J. Med.* 73: 413-419, 1982.
109. Oldham, R.K.: Biological Response Modifier Program and cancer chemotherapy. *Int. J. Tiss. Reac.* IV (3) 173-188, 1982.
110. Fidler, I.J., Berendt, M., and Oldham, R.K.: The rationale for and design of screening assays for the assessment of biological response modifier for cancer treatment. *J. Biol. Response Modif.*, 1:15-26, 1982.
111. Knost, J.A., Sherwin, S., Abrams, P., and Oldham, R.K.: Increased steroid dependence after recombinant leukocyte interferon therapy. *Lancet*. II (8258):1287-1288, 1981.



124. Fer, M.F., Sherwin, S.A., Oldham, R.K., Greco, F.A., Mathews, M.J.: Poorly differentiated lung cancer. *In* Fer, M.F., Greco, F.A., and Oldham, R.K. (Eds): *Poorly Differentiated Neoplasms and Tumors of Unknown Origin, Seminars in Oncology*, Vol.9, No.4, New York, Grune and Stratton, 456-466, 1982.
125. Key, M.E., Bernhard, M.I., Hoyer, L.C., Foon, K.A., Oldham, R.K., and Hanna, Jr., M.G.: Guinea pig 10 hepatocarcinoma model for monoclonal antibody serotherapy: *In vivo* localization of a monoclonal antibody in normal and malignant tissues. *J. Immunol.*, 139: 1451-1457, 1983.
126. Foon, K.A., Bernhard, M., Oldham, R.K.: Monoclonal antibody therapy: assessment by animal tumor models. *J. Biol. Response Modif.*, 1: 277-304, 1982.
127. Fer, M.F., McKinney, T.D., Richardson, R.L., Hande, K.R., Oldham, R.K., Greco, F.A.: Cancer and the Kidney. Renal Complications of Neoplasms. *In* Clark, Cumley, Kickey (Eds): *1982 Year Book of Cancer* Chicago, Yearbook Medical Publishers 286-288, 1983.
128. Oldham, R.K. and Smalley, R.V.: Immunotherapy. The old and the new. *J. Biol. Response Modif.*, 2: 1-37, 1983.
129. Oldham, R.K.: Guest Editorial: Biological Response Modifiers. *J. Natl. Cancer Inst.*, 70: 789-796, 1983.
130. Oldham, R.K.: Biological Response Modifiers Program. *In* Hadden, J.W., Chedid, P., Spreafico, F., Willoughby, D. (Eds): *Advances in Immunopharmacology 2*, London, Pergamon Press, pp. 655-660, 1982.
131. Sherwin, S.A., Mayer, D., Ochs, J.J., Abrams, P.G., Knost, J.A., Foon, K.A., Fein, S. and Oldham, R.K.: Recombinant leukocyte A interferon in advanced breast cancer; Results of a Phase II efficacy trial. *Annals Int. Med.*, 98: 598-602, 1983.
132. Herberman, R.B. and Oldham, R.K.: Cell-mediated cytotoxicity against human tumors: Lessons learned and future prospects. *J. Biol. Response Modif.*, 2: 111-120, 1983.
133. Oldham, R.K.: Toxic effects of interferon. *Science*, 219: 902, 1983.
134. Oldham, R.K.: Biologicals: New horizons in pharmaceutical development. *J. Biol. Response Modif.*, 2: 199-206, 1983.
135. Maluish, A.E., Ortolano, J.R., Conlon, J.C., Sherwin, S.A., Leavitt, R., Strong, D.M., Weimik, P., Oldham, R.K. and Herberman, R.B.: Depression of natural killer cytotoxicity following *in vivo* administration of recombinant leukocyte interferon. *J. Immunol.*, 131: 503-507, 1983.
136. Oldham, R.K.: Monoclonal antibodies as anticancer agents. *In* Klein, T., Spector, S., Szentivanyi, A., and Friedman, H. (Eds): *Biological Response Modifiers in Human Oncology and Immunology*, New York, Plenum Press, 45-58, 1983.

- 137 Knost, J.A., Sherwin, S.A., Abrams, P.G., Ochs, J.J., Foon, K.A., Williams, R., Tuttle, R. and Oldham, R.K. The treatment of cancer patients with human lymphoblastoid interferon: A comparison of two routes of administration. *Cancer Immunol. and Immunother.*, 15:144-148, 1983.
- 138 Stevenson, H.C., Berman, J.A., and Oldham, R.K. The design of a cytopheresis program for cancer immunology research. *Plasma Therapy*, 4:57-63, 1983.
- 139 Hanna, Jr., M.G., Key, M.E., Oldham, R.K. Biology of cancer therapy: Some new insights into adjuvant treatment of metastatic solid tumors. *J. Biol. Response Modif.*, 4:295-309, 1983.
- 140 Bernhard, M.I., Foon, K.A., Oeltmann, T.N., Key, M.E., Hwang, K.M., Clarke, G.C., Christensen, W.L., Hoyer, L.C., Hanna, Jr., M.G. and Oldham, R.K. Guinea pig line 10 hepatocarcinoma model: Characterization of monoclonal antibody and in vivo effect of unconjugated antibody and antibody conjugated to diphtheria toxin A chain. *Cancer Res.* 43:4420-4428, 1983.
- 141 Oldham, R.K. Biological Response Modifiers Program. *Cancer Treat. Symposia*, 1:19-27, 1983.
- 142 Thurman, G.B., Stull, H.B. and Oldham, R.K.: The effects of human interferon on the migration of guinea pig peritoneal exudate cells. In Oppenheim, J.J. and Cohn, S. (Eds): *Interleukines, and Cytokines*, New York, Academic Press, 277-286, 1983.
- 143 Oldham, R.K. Current status of monoclonal antibodies in cancer therapy. *Clinical Immunology Newsletter* 14:132-136, 1983.
- 144 Abrams, P.G., Knost, J.A., Clarke, G., Wilburn, S., Oldham, R.K. and Foon, K.A.: Determination of the optimal human cell lines for human hybridomas. *J. Immunol.* 131:1201-1204, 1983.
- 145 Oldham, R.K., Monoclonal antibodies in cancer therapy. *J. Clin. Onc.* 1:582-590, 1983.
- 146 Oldham, R.K.: Biological Response Modifiers Program: Subcommittee report. *J. Natl. Cancer Inst. Monograph* 63:235-247, 1983.
- 147 Smalley, R.V. and Oldham, R.K. Interferon as a biological response modifying agent in clinical trials. *J. Biol. Response Modif.* 2:401-408, 1983.
- 148 Maluish, A.E., Ortaldo, J.R., Sherwin, S.A., Oldham, R.K., and Herberman, R.B.: Changes in immunological function in patients receiving natural leukocyte interferon. *J. Biol. Response Modif.* 2:418-427, 1983.
- 149 Maluish, A.E., Leavitt, R., Sherwin, S.A., Oldham, R.K. and Herberman, R.B. Effects of recombinant alpha interferon on immune function in cancer patients. *J. Biol. Response Modif.* 2:470-481, 1983.
- 150 Bernhard, M.I., Hwang, K.M., Foon, K.A., Keenan, M., Kessler, R.M., Frincke, J.M., Tallam, D.J., Hanna, Jr., M.G., Peters, L., Oldham, R.K. Localization of <sup>111</sup>In- and <sup>125</sup>I-labeled monoclonal antibodies in guinea pigs bearing line 10 hepatocarcinoma tumors. *Cancer Res.*, 43:4429-4433.

- 1983-
151. Akiyama, Y., Miller, P.J., Thurman, G.D., Neubauer, R.H., Oliver, C., Favilla, T., Berman, J.A., Oldham, R.K. and Stevenson, H.C.: Characterization of a human blood monocyte subset with low peroxidase activity. *J Clin Invest.* 72:1093-1105, 1983.
  152. Weinstein, J.N., Steller, M.A., Keenan, A.M., Covell, D.G., Key, M.E., Sieber, S.M., Oldham, R.K., Hwang, K.M. and Parker, R.J.: Monoclonal antibodies in the lymphatics: Selective delivery to lymph node metastases of a solid tumor. *Science*, 222:423-426, 1983.
  153. Stevenson, H.C., Miller, P., Akiyama, Y., Favilla, T., Berman, J.A., Herberman, R.B., Stull, H., Thurman, G., Maluish, A., and Oldham, R.K.: A system for obtaining large numbers of cryopreserved human monocytes purified by leukapheresis and counter-current centrifugation elutriation (CCE). *J Immunol. Methods*. 62:353-363, 1983.
  154. Oldham, R.K.: Biologicals and biological response modifiers: The fourth modality of cancer treatment. *Cancer Treat. Rep.* 68:221-232, 1984.
  155. Foon, K.A., Bunn, P.A., Schroff, R.W., Mayer, D., Ihsu, S-M., Sherwin, S.A. and Oldham, R.K.: Monoclonal antibody therapy of chronic lymphocytic leukemia and cutaneous T-cell lymphoma: Preliminary observations. In Boss B.D., Langman, R.E. and Trowbridge, I.S. and Dulbecco, R. (Eds.) *Monoclonal Antibodies and Cancer*. New York, Academic Press. pp. 39-52, 1983.
  156. Foon, K.A., Abrams, P.G., Rossio, J.L., Knost, J.A. and Oldham, R.K.: Human hybridomas: Comparison of human cell lines for production of human hybridomas and development of human hybridomas producing antigen-specific IgG using *in vivo* immunized peripheral blood cells as fusing partners. In Boss, B.D., Langman, R.E. and Trowbridge, I.S. and Dulbecco, R. (Eds.) *Monoclonal Antibody and Cancer*. New York, Academic Press. pp. 143-155, 1983.
  157. Oldham, R.K.: NK cells: Artifact to reality, an odyssey in biology. *Can Metas. Rev.*, 2:232-336, 1983.
  158. Talmadge, J.E., Oldham, R.K. and Fidler, I.J.: Practical considerations for the establishment of a screening procedure for the assessment of biological response modifiers. *J. Biol. Response Modif.*, 3:88-109, 1984.
  159. Oldham, R.K., Morgan, A.C., Woodhouse, C.S., Schroff, R.W., Abrams, P.G. and Foon, K.A.: Monoclonal antibodies in the treatment of cancer: Preliminary observations and future prospects. *Med Onc and Tumor Pharm.* 1:51-62, 1984.
  160. Smalley, R.V., Foon, K.A., Schroff, R.W., Woodhouse, C., Morgan, A.C. and Oldham, R.K.: Monoclonal antibody therapy NCI (BRMP) supported trials in humans and guinea pigs. In Herberman, R.B., Micksche, M. and Smalley, R.V. (Eds.). *Proc. 13th International Congress of Chemotherapy*, Alan R. Liss Inc., New York, N.Y., 35-45, 1983.
  161. Smalley, R.V., Long, C., Sherwin, S.A. and Oldham, R.K.: Biological response modifiers. Current status and prospects as anticancer agents. In Herberman, R.B. (Eds.): *Basic and Clinical tumor Immunology*. Netherlands, Martinus Nijhoff Pub. pp. 257-300, 1983.

- 162 Smalley, R.V., Talmadge, J., Oldham, R.K., and Thurman, G.B.: The thymosins: preclinical and clinical studies with fraction V and alpha-1. *Cancer Treat. Rev.* 11:69-84, 1984.
- 163 Oldham, R.K. and Smalley, R.V.: Gamma Interferon: Early studies and potential applications. *Clin. Immunol. Newsletter* 5:69-73, 1984.
- 164 Talmadge, J.E., Maluish, A.E., Collins, M., Schneider, M., Herberman, R.R., Oldham, R.K., Wiltrout, R.H.: Immunodulation and anti tumor effects of MVE-2 in mice, *J. Biol. Response Modif.* 3:634-652, 1984.
- 165 Abrams, P.G., Ochs, J.J., Giardina, S.L., Morgan, A.C., Wilburn, S.B., Wilt, A.R., Oldham, R.K. and Foon, K.A.: Production of large quantities of human immunoglobulin in the peritoneum athymic mice: Implications for the development of anti-human idiotype monoclonal antibodies. *J. Immunol.* 132:1611-1613, 1984.
- 166 Stevenson, H.C., Schlick, E., Griffith, R., Chirigos, M.A., Brown, R., Conlon, J., Kanapa, D.J., Oldham, R.K. and Miller, P.: Characterization of biological response modifier release by human monocytes cultured in suspension in serum free medium. *J. Immunol. Meth.* 70:245-255, 1984.
- 167 Fei, M.P., Benman, J., Stevenson, H., Maluish, C., Delawter, T., Foon, K.A., Herberman, R., Oldham, R.K., with Terman, D., Young, J., and Daskal, Y.: A trial of autologous plasma perfusion over protein A in patients with breast cancer. *J. Biol. Response Modif.* 3:352-358, 1984.
- 168 Francisco, R., Omura, G.A., Birch, R., Krauss, S., Oldham, R.K.: Randomized phase III comparison of three doxorubicin based chemotherapy regimens in advanced non-small cell lung cancer. *J. Clin. Onc.* 2:391-395, 1984.
- 169 Smalley, R.V. and Oldham, R.K.: Biological Response Modifiers: Preclinical evaluation and clinical activity. *CRC Critical Reviews in Oncology/Hematology* 1:259-280, 1984.
- 170 Thurman, G.B., Stull, H.B., Miller, P.J., Stevenson, H.C. and Oldham, R.K.: Utilization of purified human monocytes in the agarose droplet assay for measuring migration inhibitory factors. *J. Immunol. Methods.* 65:41-53, 1984.
- 171 Talmadge, J.E., Lenz, B.F., Collins, M.S., Uithoven, K.A., Schneider, M.A., Adams, J.S., Pearson, J.W., Agee, W.J., Fox, R.E. and Oldham, R.K.: Tumor models to investigate the therapeutic efficiency of immunomodulators. Symposium on Models and Methods in the Immunotherapy and Chemotherapy of Cancer. *Behring Inst. Min* 74:219-229, 1984.
- 172 Oldham, R.K.: Introduction, Symposium on ex vivo plasma immunoabsorption and protein A in cancer therapy. *J. Biol. Response Modif.* 3:229-230, 1984.
- 173 Foon, K.A., Sherwin, S.A., Bunn, P.A., Longo, D. and Oldham, R.K.: Recombinant leukocyte interferon in the treatment of non-Hodgkin's lymphoma, chronic lymphocytic and cutaneous T-cell lymphoma. In *Interferon: Research, Clinical Application, and Regulatory Consideration*, Zoon, K.A., Noguchi, P.C., and Lui, T.Y. (Eds.) Elsevier Science Publishing pp 219-227, 1984.

CERTIFICATE OF MAILING BY "FIRST CLASS MAIL"

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to:  
Assistant Commissioner for Patents, Washington, D.C. 20231, on August 10, 1994.

8-27-97  
Date

Alexandra M. Parsons  
Alexandra M. Parsons

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Lynn E. Spitzer et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

DECLARATION OF LYNN E. SPITZER, M.D.  
PURSUANT TO 37 C.F.R. § 1.132

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, Lynn E. Spitzer, declare as follows:

1. I am a coinventor of the subject matter in the above-referenced application. I have been a practicing immunologist for many years. A copy of my *curriculum vitae* is attached hereto as Exhibit A.

2. I have been managing clinical trials of OncoVax<sup>TM</sup> prostate cancer vaccine which is recombinant human prostate specific antigen (rhPSA) formulated in liposomes with lipid A as adjuvant. Lipid A is a preferred adjuvant because of its ability to stimulate cell-mediated immunity in general and production of cytotoxic T cells (CTL) in particular, as opposed to alum which enhances the antibody response.

3. We conducted four clinical Phase VII trials with 4 or 5 patients in each trial. In the first two trials, eligible patients had surgically incurable and hormone-resistant prostate cancer as

do-83047

well as intact immune responses as measured by ability to respond to recall antigens. Patients were given 1 ml of the vaccine containing 100 µg rh<sup>PSA</sup> ~~PSA~~ <sup>25</sup> and 200 µg Lipid A. In the first trial, the vaccine was administered intramuscularly in two divided doses on two extremities with intact regional lymph nodes every 30 days times 3. In this trial, one patient showed skin test sensitivity to PSA with a diameter of erythema of 13 mm, and a diameter of induration of 10 mm (when diameters of more than 5 mm are considered positive responses).

4. In the second trial, the same dosage and regimen was used except that an intravenous route was employed. Again, one of five patients showed reactivity to a skin test with PSA with a diameter of erythema of 10 mm.

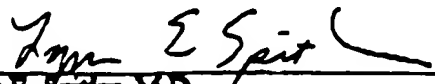
5. In a third trial, the adjuvant GM-CSF was added to the protocol and the vaccine was administered with the same protocol intramuscularly. In this test, all <sup>4 evaluable</sup> ~~8~~ <sup>25</sup> patients showed skin test reactivity to PSA with a mean diameter of erythema of 37 mm. One patient had an erythema diameter of 70x50 mm.

6. In the fourth trial, patients were treated with cyclophosphamide to diminish suppressor cell activity and the vaccine was administered intracutaneously with the addition of *Bacillus Calmette-Guérin* (BCG) at the site of vaccine administration. In this trial, 4 of the 5 patients showed a skin response to PSA with a mean diameter of erythema of 17 mm and a mean induration diameter of 9 mm.

7. The data are shown in detail on attached Exhibit B. These results show that administration of OncoVax<sup>TM</sup> is successful in eliciting a cellular response in these patients, especially in the presence of a suitable adjuvant.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Tiburon, California on 26 August 1997, by

  
Lynn E. Spitzer, M.D.

2

Serial No. 08/288,057  
Docket No. 204372008320

dc-89047

\*\*\* TOTAL PAGE. 08 \*\*\*

## CURRICULUM VITAE

LYNN E. SPITLER, M.D.

1895 Mountain View Drive  
Tiburon, California 94920

(415) 435-9861  
FAX (415) 435-6851

Social Security #366-38-1697

### Education:

University of Michigan, Ann Arbor, Michigan	1956-1959
University of Michigan Medical School Ann Arbor, Michigan M.D. (cum laude)	1959-1963

### Training:

Intern	Highland Alameda County Hospital Oakland, California	1963-1964
Resident	University of California School of Medicine San Francisco, California	1964-1966
Research Fellow	H.S. Lawrence, M.D. Department of Medicine - Immunology New York University New York, New York	1966-1967
Immunology Trainee	H. Hugh Fudenberg, M.D. University of California School of Medicine San Francisco, California	1967-1969

**Teaching Appointments and Employment:**

Instructor of Medicine in Residence University of California School of Medicine San Francisco, California 94143	1970-1971
Assistant Professor of Medicine in Residence University of California School of Medicine San Francisco, California 94143	1971-present
Research Associate Cancer Research Institute University of California School of Medicine San Francisco, CA 94143	1971-1978
Director, Melanoma Center Northern California Health Center San Francisco, California 94118	1978-1990
Director of Research Children's Hospital of San Francisco San Francisco, California 94118	1978-1981
Member, Graduate Group in Comparative Pathology Department of Comparative Pathology University of California, Davis Davis, California	1976-1981
Senior Vice President XOMA Corporation 2910 Seventh Street Berkeley, California 94710	1981-1988
Associate Scientific Director Biotherapeutics, Inc. 357 Riverside Drive Franklin, Tennessee, 37065-1676	1988-1989



Director  
Northern California Melanoma Centers  
1895 Mountain View Drive  
Tiburon, California 94920

1990-present

President  
Jenner Technologies  
1895 Mountain View Drive  
Tiburon, California 94920

1991-present

**Awards and Honors:**

Recipient: Dernham Senior Fellowship  
California Div. of the American Cancer Society

1969-1971

Recipient: Research Career Development Award  
National Institutes of Health

1971-1976

Alpha Omega Alpha (Junior year)

1961

Outstanding Young Women of America

1968

Who's Who of American Women

1972

Who's Who in the West

1973

**Board Certification:**

American Board of Internal Medicine  
American Board of Allergy and Immunology

1972

1974

**Licensure:**

Michigan 25985  
New York 96454  
California C-26446

**Memberships in Professional Societies:**

American Association of Immunologists  
American Association for the Advancement of Science  
Alpha Epsilon Iota  
Western Society for Clinical Research  
American Federation of Clinical Research  
Society of Biological Therapy  
American Association for Cancer Research

**Patents:**

Patent #4,489,810 for "Composition and Method for Transplantation Therapy"  
  
Patent #4,590,071 for "Human Melanoma Specific Immunotoxins"

**PUBLIC SERVICE**

**National Review Committees:**

Allergy and Immunology Research Committee, NIAID, NIH	1976-1980
Merit Review Board in Immunology, VA, Washington, D.C. (Chairman 1979-1980)	1976-1980

**Editorial Boards:**

The Journal of Immunology	1975-1978
The International Journal of Immunopharmacology	1979-1984
Immunologia Clinica e Sperimentale	1982-1986
Antibody Immunoconjugates and Radiopharmaceuticals (Associate Editor)	1987-present
Molecular Biotherapy	1987-present
Cancer Biotherapy	1991-present

### Manuscript Reviews:

American Review of Respiratory Disease  
Annals of Allergy  
Annals of Internal Medicine  
Archives of Dermatology  
Archives of Internal Medicine  
California Medicine  
Cancer  
Cancer Immunology and Immunopathology  
Cancer Research  
Cellular Immunology  
Chest  
Infection and Immunity  
Infectious Disease and Immunology  
International Journal of Immunopharmacology  
Journal of Clinical Investigation  
Journal of Immunology  
Journal of Infectious Diseases  
Journal of the American Academy of Dermatology  
Molecular Biotherapy  
Nature  
New England Journal of Medicine  
Science  
The Western Journal of Medicine

### Special Consultants:

National Institutes of Health Grant Reviews  
National Institutes of Health Site Visits  
United States Tuberculosis Panel Task Group  
Atomic Energy Commission Site Visits  
Public Education Panel, National Multiple Sclerosis Society  
Review of Grant Applications for the National Science Foundation  
Enterprise for High School Students, Medical Apprenticeship Program,  
San Francisco, California  
Board of Directors, San Francisco Unit, American Cancer Society  
Research and Human Experimentation Committee, Children's Hospital  
of San Francisco  
U.S. Energy Research Development Administration Site Visits

## BIBLIOGRAPHY

(Excluding Abstracts)

LYNN E. SPITLER, M.D.

1. Spidler L.E., Cochrum K.C., and Fudenberg H.H.: Mycoplasma inhibition of phytohemagglutinin stimulation of lymphocytes. Science 161:1148-1149, 1968.
2. Spidler L.E., Huber H., and Fudenberg H.H.: Inhibition of capillary migration by antigen-antibody complexes. J. Immunol. 102:404-411, 1969.
3. Cochrum K.D., Spidler L.E., Najarian J.S., and Fudenberg H.H.: A new source of large numbers of lymphocytes and studies on their culture. In Proceedings of the Third Annual Leukocyte Culture Conference. Edited by W.O. Rieke, Appleton-Century-Crofts, New York, New York, 1969, pp. 169-175.
4. Spidler L.E., and Lawrence H.S.: Studies of lymphocyte culture; products of sensitive lymphocyte-antigen interaction. J. Immunol. 103:1072-1077, 1969.
5. Spidler L.E., Benjamini E., Young J.D., Kaplan H., and Fudenberg H. H.: Studies on the immune response to a characterized antigenic determinant of the tobacco mosaic virus protein. J. Exp. Med. 131:133-148, 1970.
6. Spidler L.E., and Fudenberg H.H.: Multiple antibody response following busulfan therapy. Vox Sang. 18:450-458, 1970.
7. Spidler L.E., and Fudenberg H.H.: Products of interaction of antigen-sensitive leukocytes and antigen: further characterization of the mitogenic factor. J. Immunol. 104:544-549, 1970.
8. Levin A.S., Spidler L.E., Sites D.P., and Fudenberg H.H.: Wiskott-Aldrich syndrome: a genetically determined cellular immunological deficiency. Clinical and laboratory responses to therapy with transfer factor. Proc. Natl. Acad. Sci. 67:821-827, 1970.
9. Spidler L.E., Keuppers F., and Fudenberg H.H.: Normal macrophage function in pulmonary alveolar proteinosis. Am. Rev. Resp. Dis. 102:975-977, 1970.

10. Spidler L.E., Levin A.S., Huber H., and Fudenberg H.H.: Prediction of results of transfer factor therapy in the Wiskott-Aldrich syndrome by monocyte IgG receptors. In Proceedings of the Sixth Leukocyte Culture Conference. Edited by M.R. Schwartz. Academic Press, Inc., New York and London, 1972, pp. 795-803.
11. Henderson W.R., Fukuyama K., Epstein W.L., and Spidler L.E.: In vitro demonstration of delayed hypersensitivity in patients with berylliosis. J. Invest. Dermatol. 58:5-8, 1972.
12. Senyk G., Nitecki D.E., Spidler L.E., and Goodman J.W.: The immune response to glucagon in conjugated form. Immunochimistry 9:79-110, 1972.
13. Henderson W.R., Fukuyama K., Epstein W.L., and Spidler L.E.: Demonstration of cellular immunity to tumor-specific antigens of malignant melanoma in hamsters by inhibition of macrophage migration. J. Invest. Dermatol. 58:299-332, 1972.
14. Spidler L.E., Von Muller C., Fudenberg H.H., and Eylar E.A.: Experimental allergic encephalitis: dissociation of cellular immunity to brain protein and disease production. J. Exp. Med. 136:156-174., 1972.
15. Fudenberg H.H., Spidler L.E., and Levin A.S.: Treatment of immune deficiency. Ann. N.Y. Acad. Sci. 230:529-535, 1972.
16. Hitzig W.H., Font Anellaz H.P., Muntener U., Paul S., Spidler L.E., and Fudenberg H.H.: Transfer factor: immunologische Grundlagen und therapeutische Erfahrungen. Schweizerische Medizinische Wochenschrift 102:1237, 1972.
17. Spidler L.E., Levin A.S., Sites D.P., Fudenberg H.H., Pirofsky B., August C.S., Steinhilber E.R., Hitzig W.H., and Gatt R.A.: The Wiskott-Aldrich syndrome: results of transfer factor therapy. J. Clin. Invest. 51:3216-3224, 1972.
18. Lieber E., Hsu L., Spidler L.E., and Fudenberg H.H.: Cytogenetic findings in a parent of a patient with Fanconi's anemia. Clin. Genet. 3:357-363, 1972.
19. Spidler L.E., Levin A.S., and Fudenberg H.H.: Human lymphocyte transfer factor. In Methods in Cancer Research, vol. 8. Edited by H. Busch, Academic Press, New York, 1973, pp. 59-106.
20. Henderson W.R., Fukuyama K., Epstein W.L., and Spidler L.E.: Blocking of a cellular immune reaction to malignant melanoma by immunoglobulin from tumor-bearing animals. J. Reticul. Soc. 13:155-160, 1973.

21. Epstein W.L., Sagebiel R., Spidler L.E., Wybran J., Reed W., and Blois M.S.: Halo nevus and melanoma. JAMA 225:373-377, 1973.
22. Mozar H.n., Finnigan P.B., Petzold H., Spidler L.E., Emmons R.W., and Rothenberg B.: Myelopathy following duck embryo rabies vaccine. JAMA 224:1605-1607, 1973.
23. Astor S.H., Spidler L.E., Frick O.L., and Fudenberg H.H.: Human leukocyte migration inhibition in agarose using four antigens: correlation with skin reactivity. J. Immunol. 110:1174-1179, 1973.
24. Levin A.S., Spidler L.E., and Fudenberg H.H.: Transfer factor therapy in immune deficiency states. Ann. Rev. Med. 24:175-208, 1973.
25. Spidler L.E., Levin A.S., and Fudenberg H.H.: Agammaglobulinemia absent delayed sensitivity and lymphopenia without infections: demonstration of immunologic unknowns. Am. J. Med. 54:371-377, 1973.
26. Bloom B.R., Ceppellini R., Cetotini J.C., David J.R., Kunkel H., Landy M., Lawrence H.S., Maini R., Nussenzweig V., Perlman P., Spidler L.E., Rosen F., and Zabriskie J.: In vitro methods of cell-mediated immunity: a progress report. Cell. Immunol. 6:331-347, 1973.
27. Levin A.S., Spidler L.E., and Fudenberg H.H.: Immune deficiency states. In Introduction to Clinical Allergy. Edited by B. Finegold, C.C. Thomas, Springfield, Illinois, 1973, pp. 346-359.
28. Hoffman P.M., Gaston D.D., and Spidler L.E.: Comparison of experimental allergic encephalomyelitis induced with spinal cord, basic protein, and synthetic encephalitogenic peptide. Clin. Immunol. and Immunopath. 1:364-371, 1973.
29. Wybran J., Levin A.S., Spidler L.E., and Fudenberg H.H.: Rose-forming cells, immunologic deficiency diseases and transfer factor. New Engl. J. Med. 288:710-713, 1973.
30. Feigin R.D., Shackelford P.G., Eisen S., Spidler L.E., Pickering L.K., and Anderson D.C.: Treatment of mucocutaneous candidiasis with transfer factor. Pediatrics 53:63-70, 1974.
31. Spidler L.E., Levin A.S., and Fudenberg H.H.: Transfer factor. In Clinical Immunobiology, vol. 2. Edited by F.H. Bach and R.A. Good, Academic Press, New York, 2:153-175, 1974.
32. Fudenberg H.H., Levin A.S., Spidler L.E., Wybran J., and Byers V.: The therapeutic uses of transfer factor. Hosp. Prac. 9:95-104, 1974.

33. Catanzaro A., Spidler L.E., and Moser K.M.: Immunotherapy of coccidioidomycosis. J. Clin. Invest. 54:690-701, 1974.
34. Valdimarsson H., and Spidler L.E. (Chairman): Transfer factor, clinical application. In Progress in Immunology II, vol. 5. Edited by L. Brent and J. Holborow. North Holland Publication Company, Amsterdam, 1974, pp. 377-382.
35. Spidler L.E., Wybran J., Lieberman R., Levinson D., Epstein W., and Hendrickson C.: Results of intra-lesional BCG therapy in malignant melanoma: clinical and immunologic evaluation and complications. In Neoplasm Immunity: BCG Vaccination, Proceedings of a Chicago Symposium. Edited by R.G. Crispen, Institute for Tuberculosis Research, University of Illinois, Chicago, 1974, pp. 45-48.
36. Spidler L.E., Von Muller C. M., and Young J.D.: Experimental allergic encephalitis, study of cellular immunity to the encephalitogenic determinant. Cell. Immunol. 15:143-151, 1975.
37. Spidler L.E.: Transfer factor. Cutis Magazine 15:420-423, 1975.
38. Levin A.S., Byers V.S., Fudenberg H.H., Wybran J., Hackett A.J., Johnston J.O., and Spidler L.E.: Osteogenic sarcoma: immunologic parameters before and during immunotherapy with tumor-specific transfer factor. J. Clin. Invest. 55:487-499, 1975.
39. Catanzaro A., Spidler L.E., and Moser K.M.: Cellular immune response in coccidioidomycosis. Cell. Immunol. 15:360-371, 1975.
40. Spidler L.E., Spath P., Cooper N., and Fudenberg H.H.: Phagocytes and C4 in paraproteinemia. Brit. J. Haemat. 29:279-292, 1975.
41. Levin A.S., Spidler L.E., and Fudenberg H.H.: Transfer factor I: methods of therapy. In Immunodeficiency in Man and Animals: Birth Defects: Original Article, Series II. Edited by D. Bergsma. Sinauer Associates, Publishers, Sunderland, Massachusetts, 1975, pp. 445-448.
42. Spidler L.E., Levin A.S., and Fudenberg H.H.: Transfer factor II: results of therapy. In Immunodeficiency in Man and Animals: Birth Defects: Original Article, Series II. Edited by D. Bergsma. Sinauer Associates, Publishers, Sunderland, Massachusetts, 1975, pp. 449-456.

43. McKhan C.F., Hendrickson C.G., Spider L.E., Gunnarsson A., Banerjee D., and Nelson W.R.: Immunotherapy of melanoma with BCG: two fatalities following intra-lesional injection. Cancer 35:514-520, 1975.
44. Hoffman P.M., Spider L.E., Hsu M., and Fudenberg H.H.: Leukocyte migration inhibition in agarose. Cell. Immunol. 18:21-30, 1975.
45. Devich K.B., Lee J.C., Epstein W.L., Spider L.E., and Hopper J., Jr.: Renal lesions accompanying poison oak dermatitis. Clin. Nephrol. 3:106-113, 1975.
46. Spider L.E., Levin A.S., Stites D.P., Fudenberg H.H., and Huber H.: The Wiskott-Aldrich syndrome, immunologic studies in nine patients and selected family members. Cell. Immunol. 19:210-218, 1975.
47. Catanzaro A., and Spider L.E.: Transfer factor in diseases of the lung. In Immunologic and Infectious Reactions in the Lung. Edited by C.H. Kirkpatrick and H.Y. Reynolds. Marcel Dekker, New York, 1976. pp. 519-548.
48. Fidler I.J., and Spider L.E.: The effects of levamisole on in vivo and in vitro murine host response to syngeneic transplantation tumor. J. Natl. Cancer Inst. 55:1107-1112, 1975.
49. Olson J.A., Nelms D.C., Silverman S., and Spider L.E.: Levamisole, a new treatment for recurrent aphthous stomatitis. Oral Surg., Oral Med., Oral Path. 41:588-600, 1976.
50. Welch T.M., Triglia R., Spider L.E., and Fudenberg H.H.: Preliminary studies on human "transfer factor" activity in guinea pigs: systemic transfer of cutaneous delayed-type hypersensitivity to PPD and SKSD. Clin. Immunol. Immunopathol. 5:407-415, 1976.
51. Hoffman P.M., Spider L.E., and Hsu M.: Leukocyte migration inhibition in guinea pigs. 1. Correlation with skin test reactivity and macrophage-migration inhibition. Cell. Immunol. 21:358-363, 1976.
52. Spider L.E., Levin A.S., and Wybran J.: Combined immunotherapy in malignant melanoma: regression of metastatic lesions in two patients concordant in timing with systemic administration of transfer factor and bacillus Calmette-Guerin. Cell. Immunol. 21:1-19, 1976.
53. Levinson A.I., Hewell P.C., Stites D.P., Spider L.E., and Fudenberg H.H.: Co-existent lymphoid interstitial pneumonia, pernicious anemia, and agammaglobulinemia. Comment on autoimmune pathogenesis. Arch. Intern. Med. 136:312-316, 1976.



54. Spider L.E.: Delayed hypersensitivity skin testing. In Manual of Clinical Immunology. Edited by N.R. Rose and H. Friedman. American Society for Microbiology, Washington, D.C., 1976, pp. 53-63.
55. Catanzaro A. and Spider L.E.: For the Coccidioidomycosis Cooperative Treatment Group: Clinical and immunologic results of transfer factor therapy in coccidioidomycosis. In Transfer Factor Basic Properties and Clinical Applications. Edited by M.S. Ascher, A.A. Godlieb and C.H. Kirkpatrick, Academic Press, New York, 1976, pp 477-491.
56. Spider L.E.: Malignant melanoma. J. Invest. Dermatol. 67:435-441, 1976.
57. Dau P.C., Johnson K.P., and Spider L.E.: The effect of levamisole on cellular immunity in multiple sclerosis. Clin. Exp. Immunol. 26:302-309, 1976.
58. Wybran J., Spider L.E., Lieberman R., and Fudenberg H.H.: "Active" T-cell rosettes and total T-rosettes in patients with melanoma following intratumoral inoculation of BCG: a clue to the mechanism of action of baccillus Calmette-Guerin? Cancer Immunol. Immunother. 1:153-156, 1976.
59. Triglia R., and Spider L.E.: Human "transfer factor" activity in guinea pigs. Further studies. In Transfer Factor. Basic Properties and Clinical Applications. Edited by M.S. Ascher, A.A. Gottlieb and C.H. Kirkpatrick, Academic Press, New York, 1976, pp. 695-703.
60. Spider L.E., and Von Muller C.: Leukocyte migration inhibition in agarose. In In Vitro Methods in Cell-Mediated and Tumor Immunity. Edited by B. Bloom and J.R. David. Academic Press, New York, 1976, pp. 645-650.
61. Stevens D.A., et al.: (Coccidioidomycosis Cooperative Treatment Group): Transfer factor therapy of infectious diseases. In Advances in Dermatopharmacology (Chapter 6). Edited by P. Frost, E.C. Gomez and N. Zaias, Spectrum Publications, 1977, pp. 67-74.
62. Spider L.E., Glogau R., Nelms D., Silverman S., Jr., Olson J., O'Conner R., Ostler H., Smolin G., Basch K., Wong P., Engleman E.P., and Brugmans J.: Clinical and immunological effects of levamisole. In Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasias. (Fogarty International Center Proceedings 28). Edited by M.A. Chirigos, U.S. Government Printing Office, Washington, D.C., 1974, pp. 71-75.
63. Ibrahim A.B, Triglia R., Dau P.C., and Spider L.E.: Anti-tumor effects of levamisole on an allogeneic hamster melanoma and a syngeneic rat hepatoma. In Progress in Cancer

Research, vol. 2. Control of Neoplasia by Modulation of the Immune System. Edited by M.A. Chirigos, Raven Press, New York, 1977.

64. Spider L.E., and Dau P.C.: Immunotherapy in infectious disease, autoimmunity and cancer. Acta Neurol. Scand. (Suppl. 63):227-233, 1977.
65. Spider L.E., Glogau R.G., Nelms D.C., Basch C.M., Olson J.A., Silverman S., Jr., and Engleman E.P.: Clinical and immunologic effects of levamisole. In Progress in Cancer Research, vol. 2. Control of Neoplasia by Modulation of the Immune System. Edited by M.A. Chirigos, Raven Press, New York, 1977, pp. 217-225.
66. Spider L.E., Littooy F.N., and Sagebiel R.W.: Cellular immunity in patients with malignant melanoma and their household contacts. Cancer Immunol. Immunother. 2:69-76, 1977.
67. Silverman S., Jr., Olson J.A., Nelms D.C., and Spider L.E.: Recurrent aphthous stomatitis: current status of etiology and treatment. J. Calif. Dental Assoc. 2/1977, pp. 38-44.
68. Wolf R.E., Fudenberg H.H., Welch T.M., Spider L.E., and Ziff M.: Treatment of Becker's syndrome with transfer factor. JAMA 238:869-871, 1977.
69. Spivack S.D., Spider L.E., and Dunphy J.E.: Oncology and cancer chemotherapy. In Current Surgical Diagnosis and Treatment, Edited by J.E. Dunphy and L.W. Way, Lange Medical Publications, Los Altos, California, 1977, pp. 1059-1082.
70. Dau P.C., Lindstrom J.M., Cassel C.K., Denys E.H., Shev E.E., and Spider L.E.: Plasmapheresis and immunosuppressive drug therapy in myasthenia gravis. New Engl. J. Med. 297:1134-1140, 1977.
71. Berkel A.I., Ersoy F., Epstein L.B., and Spider L.E.: Transfer factor therapy in ataxia-telangiectasia. Clin. Exp. Immunol. 29:376-384, 1977.
72. Basch C.M., Spider L.E., Engleman E.G., and Engleman E.P.: Cellular immune reactivity in patients with rheumatoid arthritis and effects of levamisole. J. Rheumatol. 4:377-388, 1977.
73. Spider L.E.: Transfer factor. Int. J. Dermatol. 17:445-458, 1978.
74. Spider L.E., Sagebiel R.W., Wong P.P., Malm T.M., Chase R.H., and Gonzalez R.L.: A randomized double-blind trial of adjuvant therapy with levamisole versus placebo in patients with malignant melanoma. In Progress in Cancer Research and Therapy, vol. 6.

Immunotherapy of Cancer: Present Status of Trials in Man. Edited by W.D. Terry and D. Windhorst, Raven Press, New York, 1978, pp. 73-79.

75. Spider L.E., Wong P., and Sagebiel R.: Combined immunotherapy of malignant melanoma-unusual survival following cerebral metastasis. Arch. Dermatol. 114:1501, 1978.
76. Gross P.A., Patel C., and Spider L.E.: Disseminated cryptococcus treated with transfer factor. JAMA 240:2460-2462, 1978.
77. Suquet Von Muller C., Spider L.E., and LeCocq J.: Experimental allergic encephalitis: study of cellular immunity during disease suppression. Eur. J. Immunol. 8:771-776, 1978.
78. Spider L.E.: Transfer factor. In Handbook Series in Clinical Laboratory Science. Section F: Immunology, vol. 1, part 1. Edited by A. Baumgarten and F.F. Richards, CRC Press, West Palm Beach, Florida, 1978, pp. 87-106.
79. Gonzalez R.L., Spider L.E., and Sagebiel R.W.: Effect of levamisole as a surgical adjuvant therapy for malignant melanoma. Cancer Treatment Reports 62:1703-1707, 1978.
80. Spider L.E., Mischak R., and Basch C.: Pharmacological modification of suppressor cell activity. In The Pharmacology of Immuregulation. Edited by G.H. Werner and F. Floch, Academic Press, New York, 1978, pp. 239-251.
81. Mischak R.P., Dau P.C., Gonzalez R.L., and Spider L.E.: In vitro testing of suppressor cell activity in myasthenia gravis. In International Conference on Plasmapheresis and the Immunobiology of Myasthenia Gravis. Edited by P.C. Dau; Clark, Houghton, Mifflin, New York, 1979, pp. 72-78.
82. Gonzalez R.L., Wong P., and Spider L.E.: Altered regulation of mitogen responsiveness by suppressor cells in multiple sclerosis. Clin. Exp. Immunol. 36:78-84, 1979.
83. Jonas S., Wichter M., and Spider L.E.: Amytrophic lateral sclerosis: failure of transfer factor therapy. Ann. Neurol. 67:59-66, 1979.
84. Spider L.E.: Transfer factor therapy in the Wiskott-Aldrich syndrome: results of long-term follow-up in 32 patients. Am. J. Med. 67:59-66, 1979.
85. Spivack S.D., Spider L.E., Holm D.C., and Dunphy J.E.: Oncology and cancer chemotherapy. In Current Surgical Diagnosis and Treatment (Chapter 49). Edited by S.D.

Spivack and L.W. Way, Lange Medical Publications, Los Altos, California, 1979, pp. 1073-1101.

86. Spider L.E.: Transfer factor in immunodeficiency diseases. Ann. N.Y. Acad. Sci. 332:228-235, 1979.
87. Gonzalez R.L., Wong P., and Spider L.E.: Adjuvant immunotherapy with transfer factor with melanoma metastatic to lung. Cancer 45:57-63, 1980.
88. Spider L.E.: Transfer factor: Failure to transfer reactivity in normal human subjects. Clin. Exp. Immunol. 39:708-716, 1980.
89. Spider L.E.: BCG, levamisole, and transfer factor in the treatment of cancer. In Current Cancer Immunology, Progress in Experimental Tumor Research, vol. 25. Edited by V. Richards, Karger Publications, New York, 1980, pp. 178-193.
90. Lozada F., Spider L.E., and Silverman S., Jr.: Results of immunologic testing in patients with erythema multiforme. J. Dent. Res. 59:567-572, 1980.
91. Lozada R., Spider L.E., and Silverman S., Jr.: Clinical immunologic responses to levamisole in 13 patients with erythema multiforme. Int. J. Immunopharmacol. 2:63-68, 1980.
92. Spitler L.E., Wray B.B., Mogerman S., Miller, III, J.J., O'Reilly R.J., and Lagios M.: Nephropathy in the Wiskott-Aldrich syndrome. Pediatrics, vol. 66, pp. 391-398, 1980.
93. Spider L.E., and Sagebiel R.W.: A randomized trial of levamisole versus placebo as adjuvant therapy in malignant melanoma. New Engl. J. Med. 303:1143-1147, 1980.
94. Spitler L.E.: Delayed hypersensitivity skin testing. In Manual of Clinical Immunology, 2nd edition. Edited by N.R. Rose and H. Friedman. American Society of Microbiology, Washington, D.C., 1980, pp. 200-212.
95. Owen R.L., Dau P.C., Johnson K.P., and Spider L.E.: Immunologic mechanisms in multiple sclerosis: exacerbation by type A hepatitis and by skin test antigens. JAMA 244:2307-2309, 1980.
96. Fisher G.L., Spider L.E., McNeil K.L., and Rosenblatt L.S.: Serum copper and zinc levels in melanoma patients. Cancer 47(7):1837-1844, 1981.

97. de Merieux P., Spidler L.E., and Paulus H.E.: Treatment of Bechets syndrome with levamisole. Arth. Rheu. 24(1):64-70, 1981.
98. Catanzaro A., Spidler L.E., Campbell G.D., and Moser K.M.: Transfer factor for histoplasmosis in a patient with Hodgkin's disease. Arch. Intern. Med. 141:533-537, 1981.
99. Spidler L.E., Sagebiel R.W., Allen R., Minor D., Dymott C., and Drake T.: Levamisole in the treatment of melanoma. Immunother. Human Cancer, section VI, pp. 289-291, 1982.
100. Spidler L.E., and Scott C.F.: Immunotherapy of melanoma. In Melanoma Antigens and Antibodies. Edited by R.A. Reisfeld and S. Ferrone, Plenum Press, New York and London, 1982, pp. 355-363.
101. Spidler L.E., and Scott C.F.: Lymphocytotoxic antibody in multiple sclerosis: activity against T-cell subsets and correlation with disease activity. In Clin. Exp. Immunol. 53:133-139, 1983.
102. Scott C.F., Cashman N., and Spidler L.E.: Experimental allergic encephalitis: treatment with drugs which alter CNS serotonin levels. J. Immunopharmacol. 4(3):153-162, 1982-83.
103. Levy J.A., Lee H.M., Kawahata R.T., and Spidler L.E.: Purification of monoclonal antibodies from mouse ascites eliminates contaminating infectious mouse type C virus and nucleic acids. In Clin. Exp. Immunol. 56:114-120, 1984.
104. Engelstad B., Khenigan A., and Spidler L.E.: Clinical radioimmunoimaging. In Diagnostic Radiology. Edited by A. Margulis and C. Gooding, RREF Press, San Francisco, 1985, pp. 525-532.
105. Spidler L.E.: Removal of xenotropic and ecotropic viruses by purification of monoclonal antibodies. In Abnormal Cells, New Products and Risk. (In vitro cellular and developmental biology. Monograph;n.6). Proceedings of a workshop held July 30-31, 1984 at the National Institutes of Health, Bethesda, Maryland. Edited by M.E. Hopps and J.C. Petricciani, Tissue Culture Association, Gaithersburg, MD, 1985, pp.121-134.
106. Englestad B.L., Spidler L.E., del Rio M., Ramos E.C., Rosendorf L.L., Reinhold C.E., Khenigan A., Huberty J.P., Corpuz S.W., Lee H.M., Okerlund M.D., Hattner R.S., and Scannon P.J.: Phase I immunolymphoscintigraphy with an indium 111-labeled-antimelanoma monoclonal antibody. Radiology 161:419-422, 1986.

107. Engelstad B.C., Ramos E.C. Stoudemire J., O'Connell J.W., Villanueva J., Faulkner D.B., Hamer R.S., Spider L.E., and Scannon P.: Improved immunespecificity in monoclonal radioummunoimaging using dual radionuclide color functional maps. Investigative Radiology 21:917-921, 1986.
108. Spider L.E.: Immunotoxin therapy of malignant melanoma. Med. Oncol. & Tumor Pharmacother. 3:147-152, 1986.
109. Spider L.E., del Rio M., Khenigan A., Wedel N.I., Brophy N.A., Miller L., Harkonen W.S., Rosendorf L., Lee H.M., Mischak R.P., Kawahata R.T., Stoudemire J.B., Fradkin L.B., Bautista E.E., and Scannon P.J.: Therapy of patients with malignant melanoma using a monoclonal antimelanoma antibody-ricin A chain immunotoxin. Cancer Research 47:1717-1723, 1987.
110. Harkonen S., Stoudemire J., Mischak R.P., Spider L.E., Lopez H., and Scannon P.: Toxicity and immunogenicity of monoclonal antimelanoma antibody ricin A chain immunotoxin in rats. Cancer Research 47:1377-1382, 1987.
111. Spider L.E.: Phase I clinical trials with immunotoxins. Immunoconjugates: Antibody Conjugates in Radioimaging and Therapy of Cancer. Edited by C.W. Vogel, Oxford University Press, New York, 1987, pp.290-300.
112. Hertler, R.A., Spider, L.E., and Frankel, A.E.: Humoral immune response to ricin A chain immunotoxin in patients with metastatic melanoma. Cancer Drug Delivery 4:245 - 253, 1987
113. Miller L.L, Spider L.E., Allen R.A., and Minor D.R.: A randomized, double-blind, placebo controlled trial of transfer factor as adjuvant therapy for malignant melanoma. Cancer, 61:1543-1549, 1988.
114. Spider, L.E.: Clinical Studies: Solid Tumors. Immunotoxins. Edited by Arthur E. Frankel, Martinus Nijhoff B.V., Boston, 1988 pp. 491-512.
115. Spider L.E., and Miller L.: Clinical trials of transfer factor in malignancy. J. Exp. Pathol. 3:549-564, 1988
116. Harkonen S., Scannon P., Mischak R.P., Spider L.E., Foxall C., Kennedy D., and Greenberg R.: Phase I study of a murine monoclonal anti-lipid A antibody in bacteremic and non-bacteremic patients. Antimicrobial Agents and Chemotherapy, 710-716, 1988.

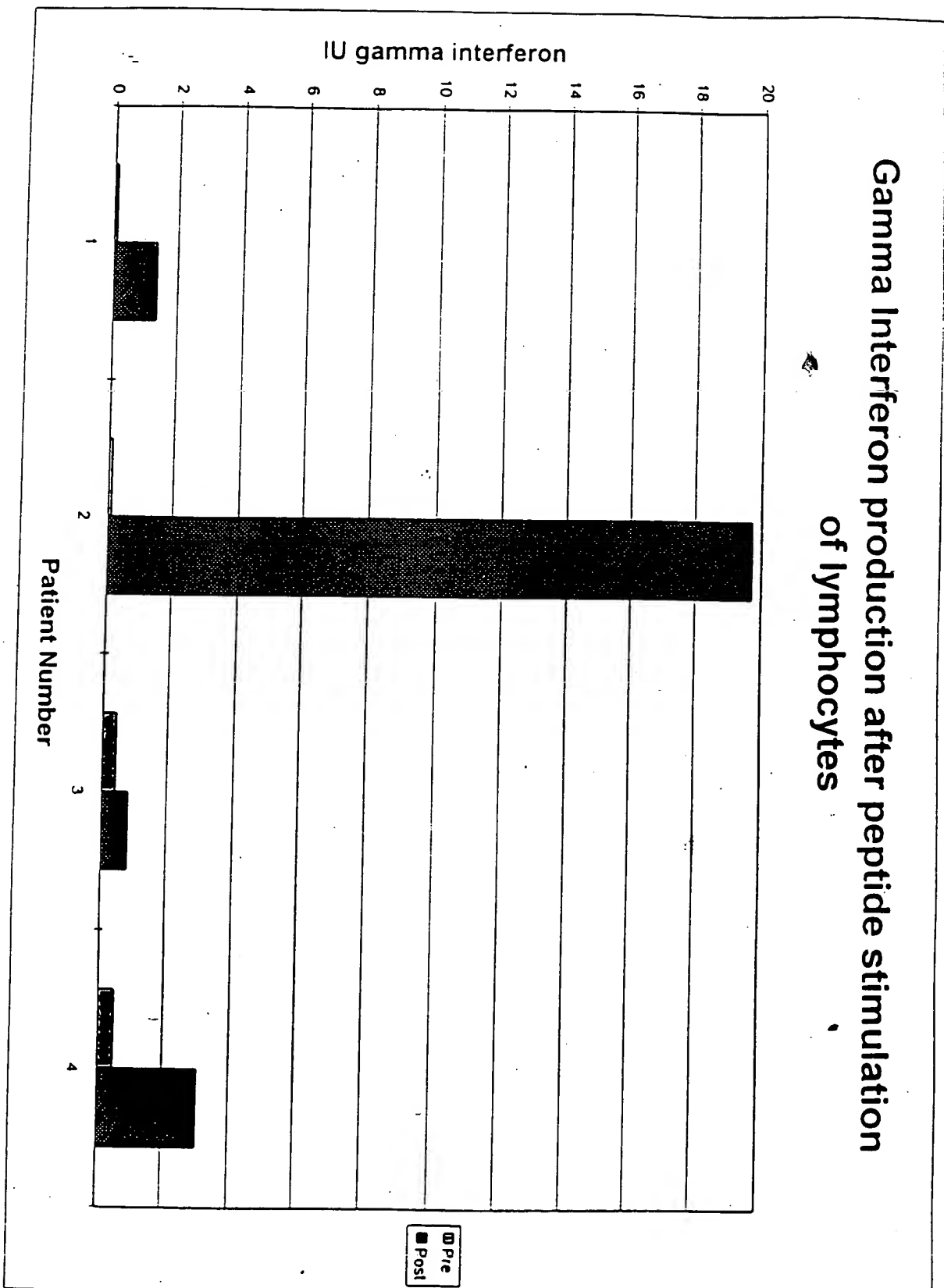
117. LoBuglio A.F., Khazaeli M.B., Lee J., Haynes A., Sumerel L., Mischak R.P., and Spider L.E. Pharmacokinetics and immune response to Xomazyme-Mel in melanoma patients. Antibody Immunoconjugates and Radiopharmaceuticals 1:305 - 310, 1988.
118. Spider, L.E., Mischak, R. and Scannon, P.: Therapy of metastatic malignant melanoma using Xomazyme-Mel, a murine monoclonal antimelanoma ricin A chain immunotoxin. Nuclear Medicine and Biology International Journal of Applied Radiation and Isotopes, Part B, 16(6):625-627, 1989.
119. Spider, L.E.: Monoclonal Antibodies In the Treatment of Malignant Melanoma. The Present and Future Role of Monoclonal Antibodies in the Management of Cancer. Frontiers of Radiation Therapy and Oncology, 24, 186-193, 1989
120. Mischak, R.P., Foxall, C., Rosendorf, L.L., Knebel, K., Scannon, P.J., Spider, L.E.: Human antibody responses to components of the monoclonal antimelanoma antibody ricin A chain immunotoxin Xomazyme [registered trademark] - MEL. Molecular Biotherapy, 2:104-109, 1990.
121. Stoudemire, J.B., Mischak, R., Foxall, C., Harkonen, W.S., Del Rio, M., and Spider, L.E.: The effects of cyclophosphamide on the toxicity and immunogenicity of ricin A chain immunotoxin in rats. Molecular Biotherapy, 2: 179-184, 1990.
122. Oratz, R., Speyer, J.L., Wernz, J.C., Hochster, H., Meyers, M., Mischak, R., and Spider, L.E.: Antimelanoma monoclonal antibody - ricin A chain immunoconjugate (XMMME-0001-RTA) plus cyclophosphamide in the treatment of metastatic malignant melanoma: Results of a phase II trial. J. Biol Response Mod., 9(4): 345-354, 1990.
123. Spider, L.E.: Immunotoxins. In: Principles of Cancer Biotherapy. Edited by R.K. Oldham, Marcel Dekker, inc., New York, pp 433-456, 1991.
124. Spider, L.E.: A randomized trial of levamisole versus placebo as adjuvant therapy in malignant melanoma. Journal of Clinical Oncology, 9: 736-740, 1991.
125. Gonzalez, R., Salem, P., Bunn Jr., P.A., Zukowski, A.A., Lamb, R., Benjamin, R.S., Spider, L., Wedel, N., and Robinson, W.A. Single-dose murine monoclonal antibody ricin A chain immunotoxin in the treatment of metastatic melanoma: a phase I trial. Mol. Biother., 3:192-196, 1991.
126. Spider, L.E., Conjugated Antibodies: Immunotoxins. In Antibody Therapy of Cancer. Edited by Robert Dillman, Marcel Dekker, New York, In Press, 1992.

127. Spidler, L.E. and Munoz, A.: Value of percentage of lymphocytes and active rosettes in determination of prognosis in malignant melanoma. In preparation, 1992.
128. Spidler, L.E., Von Wussow, P., Carey, R.W., Borden, E., Benjamin, R.S., Oratz, R., Tseng Jr., A., Mastrangelo, M., and Ernstoff, M.S.: Phase II Trial of a monoclonal antimelanoma antibody Ricin A chain immunotoxin in therapy of malignant melanoma. Journal of Clinical Oncology, in preparation.
129. Spidler, L.E., and Minor, D.R.: Monoclonal antimelanoma ricin A chain immunotoxin therapy of metastatic melanoma in an outpatient setting. Journal of Clinical Oncology, in preparation.

*Revised January, 1992*



# Gamma Interferon production after peptide stimulation of lymphocytes



# IL-4 production after peptide stimulation of lymphocytes

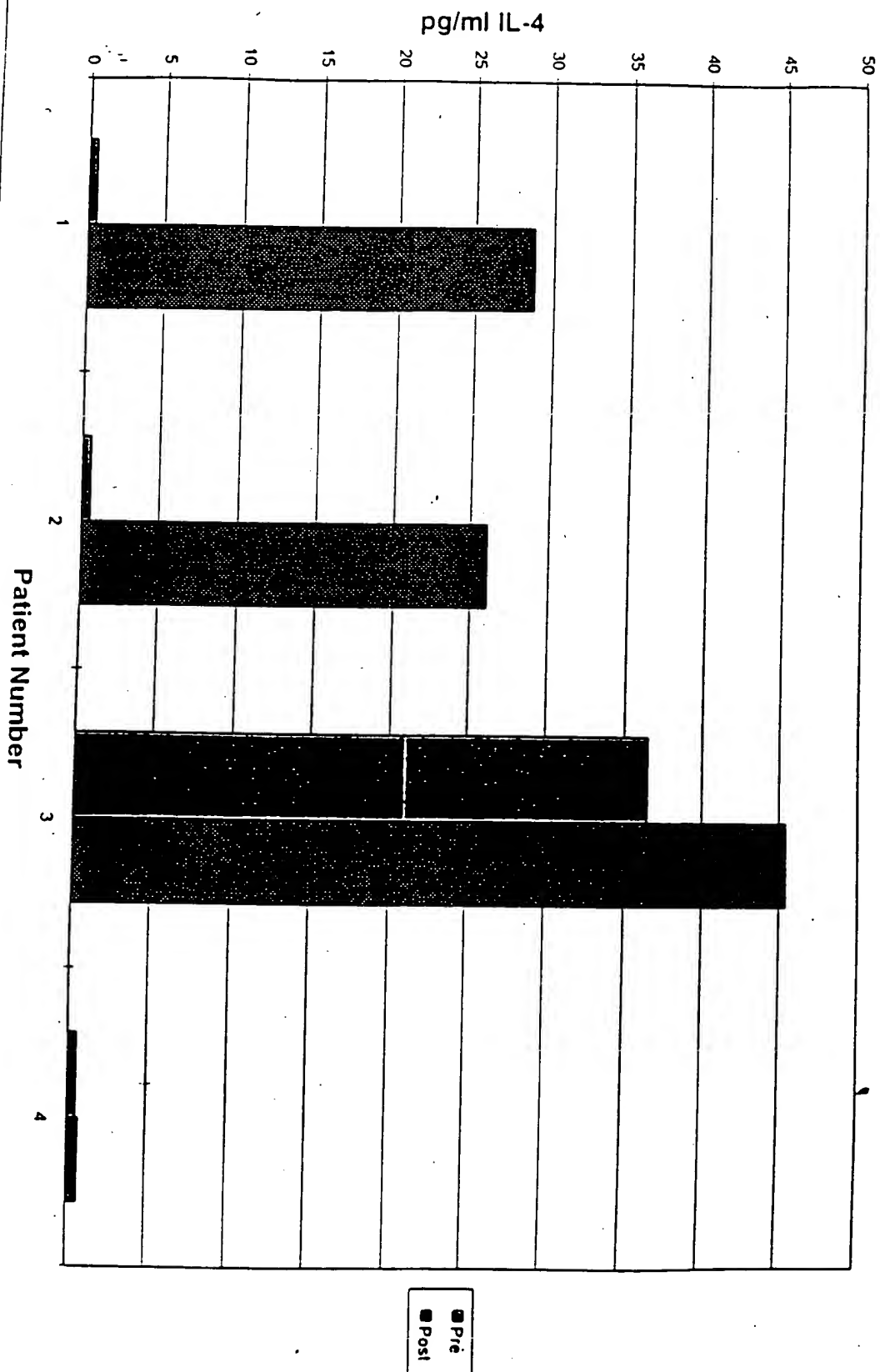


Exhibit B

# Prostate Cancer Vaccine Trials Day 90 Skin Test Results

## Clinical Trial #1 – Immunization of Patients with Prostate Cancer with OncoVax-P™ (Prostate Cancer Vaccine)

	PSA		Buffer		CEA	
	Erythema	Induration	Erythema	Induration	Erythema	Induration
001 AW	2x2	0	0	0		
002 JH	ND	ND	ND	ND		
003 MD	18x18	10x10				
004 MED	0	0	0	0		
005 HN	0	0	0	0		
006 JLB	0	0	0	0		

## Clinical Trial #2 – Intravenous Immunization of Patients with Prostate Cancer with OncoVax-P™ (Prostate Cancer Vaccine)

	PSA		Buffer		CEA	
	Erythema	Induration	Erythema	Induration	Erythema	Induration
101 RT	0	0	0	0		
102 ER	0	0	0	0		
103 MC	0	0	0	0		
104 TM	0	0	0	0		
105 AF	ND	ND	ND	ND		
106 WC	10X10	0	0	0		

## Clinical Trial #3 – Immunization of Patients with Prostate Cancer with OncoVax-P™ (Prostate Cancer Vaccine) with GM-CSF

	PSA		Buffer		CEA	
	Erythema	Induration	Erythema	Induration	Erythema	Induration
201 AM	30x20	0			0	0
202 ASP	35x30	30x30			7x7	7x7
203 CN	70x50	0			0	0
204 JA	ND	ND			ND	ND
205 GS	37x22	0			0	0

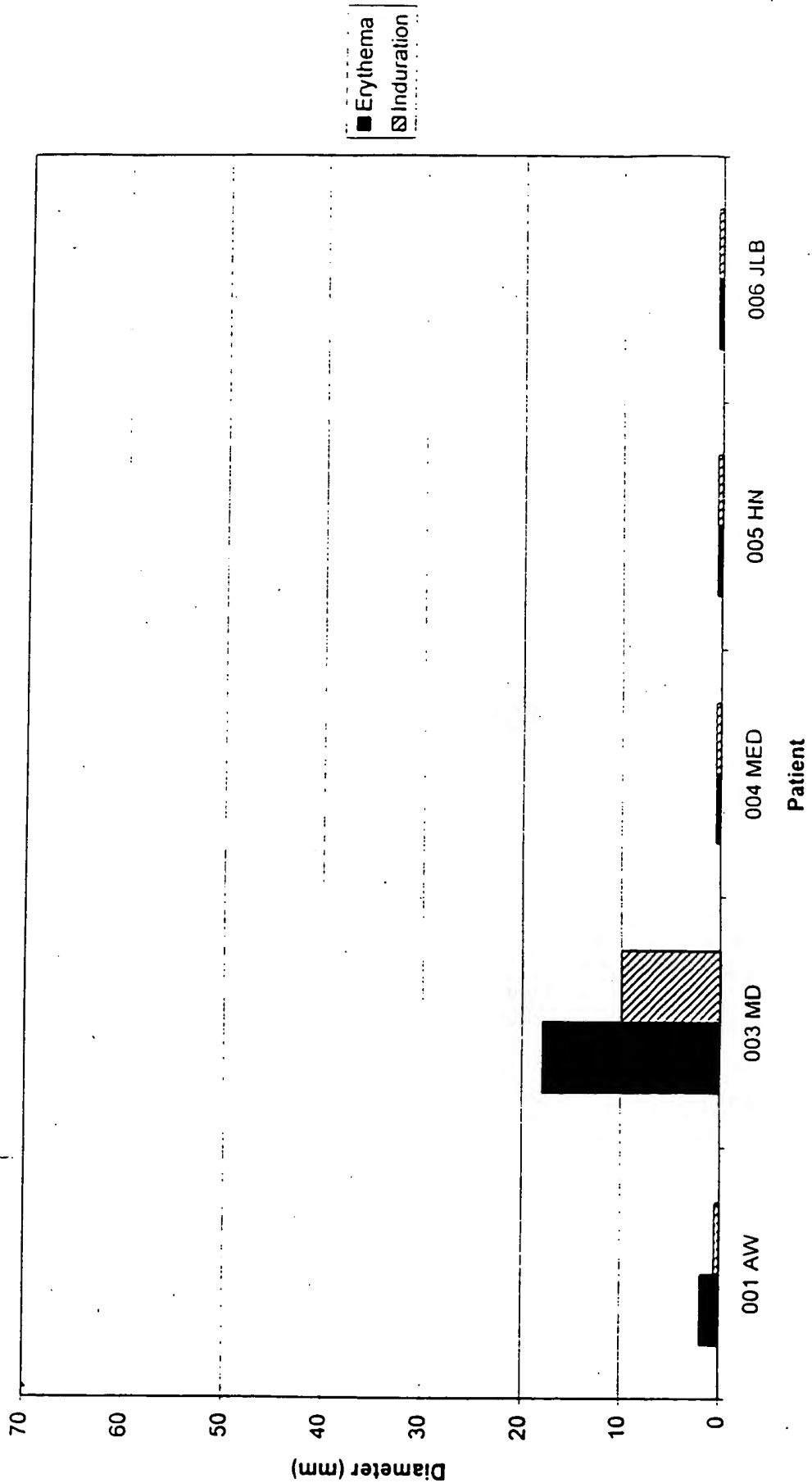
## Clinical Trial #4 – Immunization of Patients with Prostate Cancer with OncoVax-P™ (Prostate Cancer Vaccine with BCG)

	PSA		Buffer		CEA	
	Erythema	Induration	Erythema	Induration	Erythema	Induration
301 GS	32x28	32x28			0	0
302 AR	15x15	15x15			0	0
303 JE	15x12	0			0	0
304 ML	0	0			0	0
305 RD	30x30	0			0	0

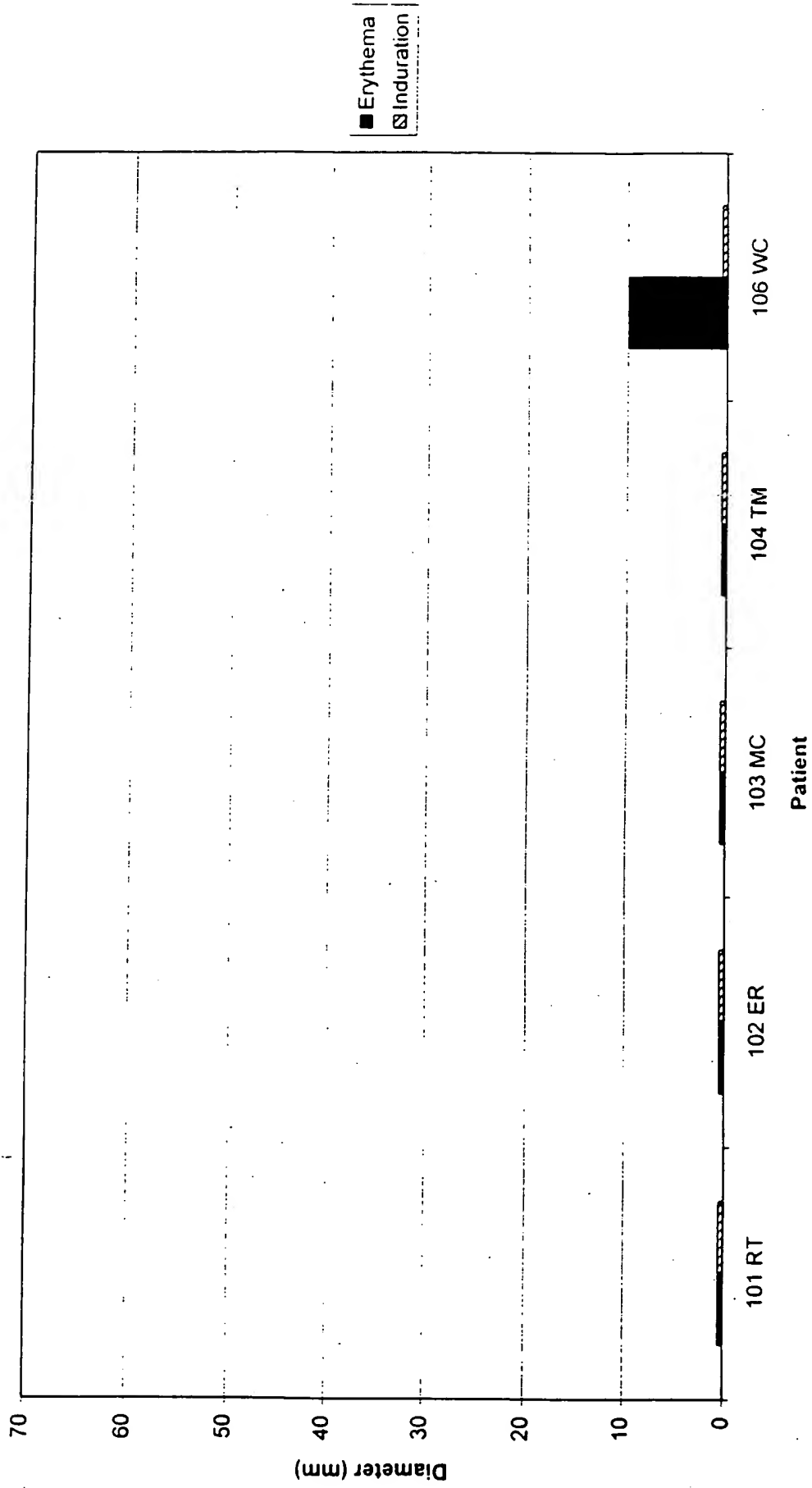
ND = Not Done

Exh. b. + B  
Spitler

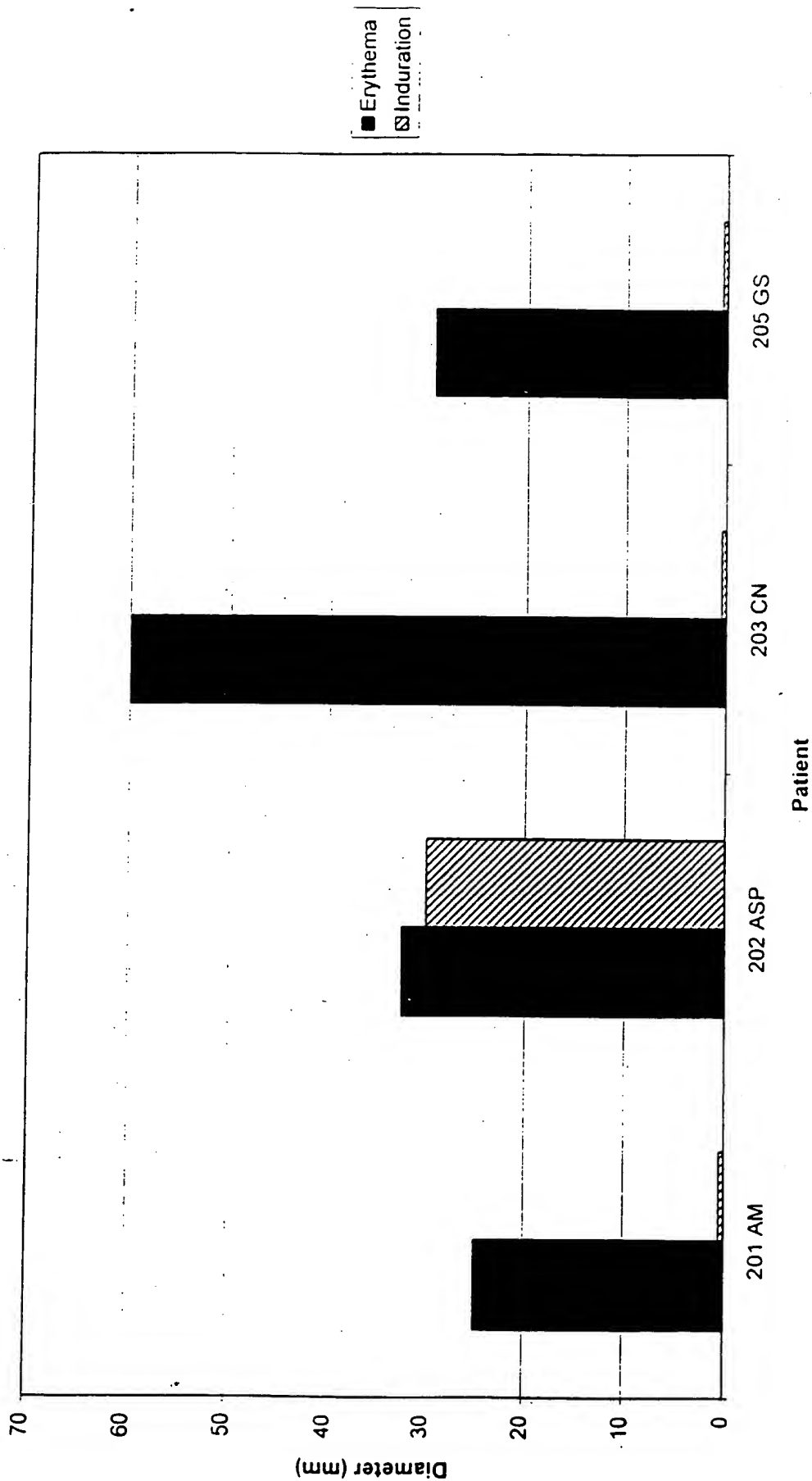
# Skin Test Reactivity to PSA Clinical Trial #1 Immunization of Patients with Prostate Cancer with OncoVax-P™



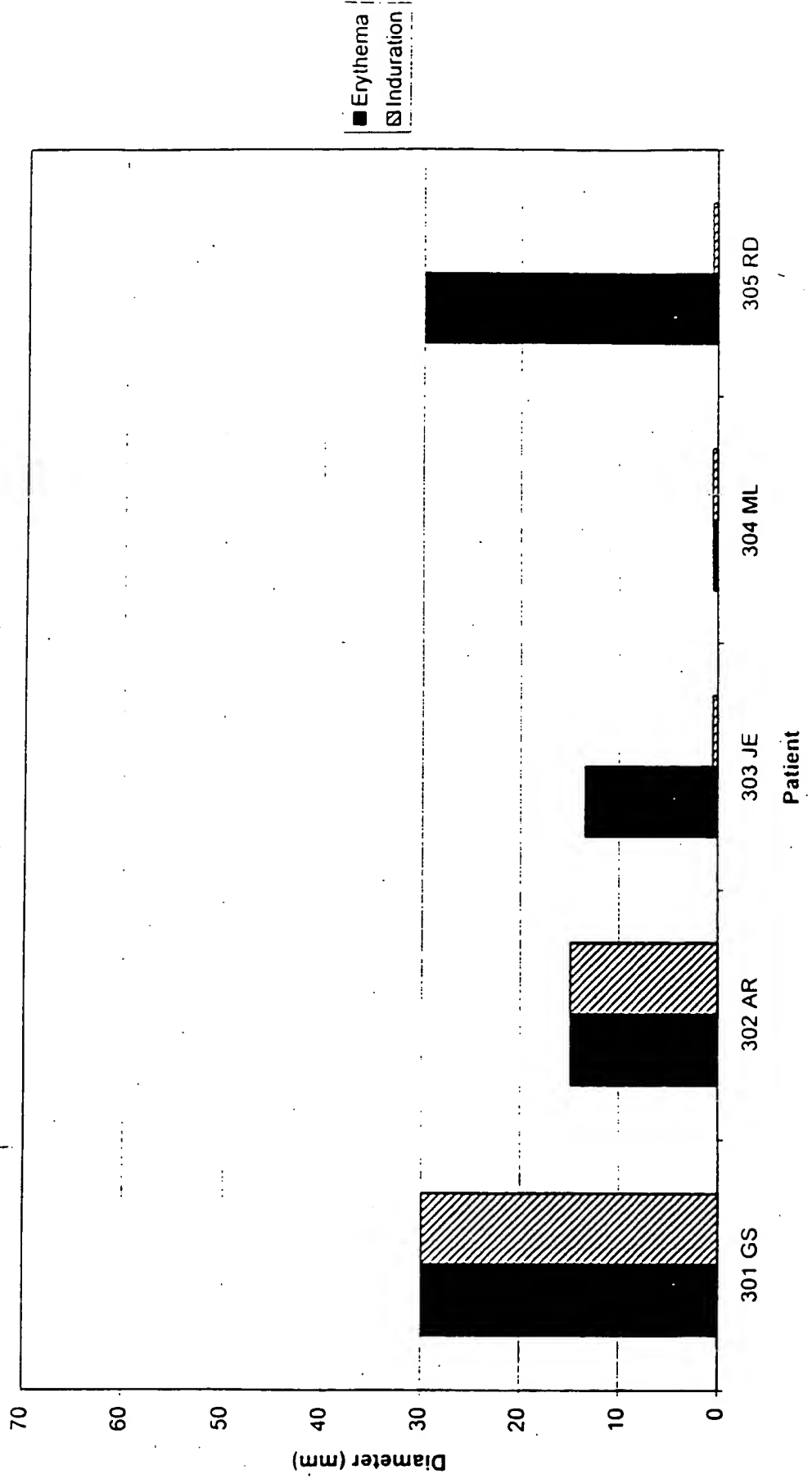
# Skin Test Reactivity to PSA Clinical Trial #2 Intravenous Immunization of Patients with Prostate Cancer with OncoVax-P™



Skin Test Reactivity to PSA  
Clinical Trial #3  
Immunization of Patients with Prostate Cancer with OncoVax-P™ with GM-CSF



Skin Test Reactivity to PSA  
Clinical Trial #4  
Intravenous Immunization of Patients with Prostate Cancer with OncoVax-P™ with BCG



PATENT  
Docket No. 204372000320

## CERTIFICATE OF MAILING BY "FIRST CLASS MAIL"

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to:  
Assistant Commissioner for Patents, Washington, D.C. 20231.

8-27-97  
DateAlexandra H. Parsons  
Alexandra H. Parsons

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Lynn E. Spitler et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

DECLARATION OF GARY R. MATYAS, PH.D.  
PURSUANT TO 37 C.F.R. § 1.132Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir: \*

1, Gary R. Matyas, declare as follows:

1. I am engaged in medical research at the Walter Reed Army Institute of Research (WAIR) and have been active in studying cellular immune responses for 2 years. A copy of my *curriculum vitae* is attached hereto as Exhibit A.

2. Under my supervision, studies were performed using Balb/C mice. In order to generate cytotoxic T lymphocytes (CTL) in response to recombinant human prostate specific antigen (rhPSA) we immunized two groups of six mice per group with OncoVax<sup>PTM</sup> alone or with OncoVax<sup>PTM</sup> mixed with aluminum hydroxide (alum, Alhydrogel, 200 µg Al<sup>+++</sup>).

dc-85046



OncoVax<sup>PTM</sup> is a liposomal formulation of rhPSA with lipid A, and the PSA dose in this protocol was 5 µg rhPSA and 20 µg lipid A. The mice were immunized at week 0 and week 4 at these dosages and euthanized at 8 weeks. The spleens were removed and spleen cells harvested, pooled and incubated for 5 days either with medium alone or in medium containing 2 µg/ml rhPSA.

3. Target cells were P815 mouse mastocytoma cells infected either with wild-type vaccinia virus or with vaccinia virus transfected with the PSA gene and the *E. coli* lacZ gene (PSAVac, Therion Biologics Corp.) at a multiplicity of infection of 10. Alternatively, the P815 cells were incubated with 10 µg/ml of the peptide CYASGWGSI which represents a potential CTL epitope at positions 153-161 of PSA.

4. After incubation for 16 hours with vaccinia viruses or peptide, the target cells were labeled with Cr<sup>51</sup> (0.1 µCi/10<sup>6</sup> cells) for 1 hour).

5. The effector cells harvested from the mouse spleens were plated in 96-well u-bottom plates and the Cr<sup>51</sup> labeled target 815 cells were added at various effector:target ratios. The plates were centrifuged at 50xg for 5 minutes and then incubated at 37°C for 5 hours. The radioactivity present in the supernatant (indicative of cytotoxic activity of the effector cells) was harvested using Skatron wicks and quantified using a gamma counter.

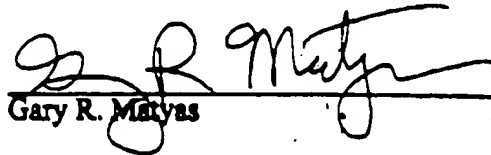
6. The attached Exhibit B shows the results. As shown, spleen cells from mice injected with either OncoVax<sup>PTM</sup> or OncoVax<sup>PTM</sup> with alum were able to lyse target cells that had been transfected with the vaccinia virus (Panels A and B). OncoVax<sup>PTM</sup> injected mice in the absence of alum (panel E) were effective in lysing the targets labeled with peptide, although when alum was included in the formulation, the resulting spleen cells were less able to do so (panel F). P815 target cells unlabeled with either peptide or vaccinia-produced PSA were not lysed (panels C, D, G, and H).

7. These results demonstrate that immunization of mice with OncoVax<sup>PTM</sup> induces lymphocytes which kill tumor cells presenting PSA antigens, indicating an antitumor response.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Washington, D.C. on 26 August 1997, by

  
\_\_\_\_\_  
Gary R. Matyas

August , 1997

## CURRICULUM VITAE

Gary R. Matyas

**Office:**

Department of Membrane Biochemistry  
Walter Reed Army Institute of Research  
Washington DC 20307-5100  
(202) 782-0875

**Home:**

18415 Snowberry Way  
Olney, MD 20832  
(301) 570-0610

**CAREER AIMS:** Research/management of membrane biochemistry laboratory with emphasis on liposome based vaccines, lipid mediators, and lipid changes during cell growth.

**PERSONAL:** Born April 30, 1956 in Berwick, PA; married, one child

**EDUCATION:**

**Graduate** Purdue University, West Lafayette, IN 47907  
Ph.D. degree; May 1985; Department of Biological Sciences  
Major Professor: D. James Morré

**Undergraduate** Pennsylvania State University, University Park, PA 16802  
B.S. degree; May 1978; Major - Biophysics

**EXPERIENCE:**

**Research** Research Chemist  
Department of Membrane Biochemistry  
Division of Biochemistry  
Walter Reed Army Institute of Research  
Washington DC 20307  
September 1988 to present  
Supervisor: Dr. Carl R. Alving

**Projects:**

- I. Development of active site monoclonal antibodies to cobra venom phospholipase A<sub>2</sub>; Inhibitors of enzyme activity

**Gary R. Matyas**

**Curriculum Vitae (continued)**

**WRAIR Projects (cont.):**

- II Mechanism of concanavalin A induced killing of mice and cultured cells
- II Incorporation of bioactive lipids into liposomes: Effect on immune response to liposome encapsulated antigens
- IV Development of monoclonal antibodies to sphingosine and sphingolipids
- V Development of a liposomal vaccine against ricin intoxication
- VI In collaboration with Jenner Technologies, development, manufacture and testing of liposomal based cancer vaccine in human clinical trials
- VII Development of a liposomal vaccine protects against Ebola virus infection through the induction of cytotoxic lymphocytes.

**Additional Duties:**

- I Division of Biochemistry Safety Officer, Division representative to WRAIR safety council; Responsibilities include: Monthly safety inspections of division laboratories; Conducting training on safety concerns; Maintaining division training records.
- II. Terminal Security Officer for the Department of Membrane Biochemistry; Duties include: Maintaining security on computer equipment in the department; Procuring of IBM compatible equipment and software.

**Staff Fellow**

**Membrane Biochemistry Section**

**Laboratory of Molecular and Cellular Neurobiology/Developmental and Metabolic Neurology Branch**

**National Institute of Neurological, Communicative Disorders and Stroke**

**National Institutes of Health**

**Bethesda, MD 20892**

**April 1985 to September 1988**

**Supervisor: Dr. Peter Fishman**

**Projects:**

- I. Glycolipid alterations induced by transfection of NIH/3T3 cells with oncogenes.
- II. Alterations of glycolipids during cell growth.

**Gary R. Matyas**

**Curriculum Vitae (continued)**

**NIH projects (cont.)**

- II Production of glycolipid crosslinking reagents for the study of the involvement of glycolipids in cell adhesion and cell growth.
- IV. The role of *ras* oncogenes in phospholipase C mediated phosphoinositide hydrolysis.

**Graduate Assistant**

Department of Biological Sciences

Purdue University

West Lafayette, IN 47907

August 1978 to April 1985

Supervisor: Dr. D. James Morré

**Projects:**

- I. Interaction of fibronectin with gangliosides.
- II Subcellular distribution and biosynthesis of gangliosides in rat liver.
- III. Loss of fibronectin and complex gangliosides in metastatic rat tumors.
- IV. Elevated levels of serum gangliosides as a means of early detection of cancer.
- V. Cytochemical localization of glycosyltransferases.

**Research Assistant**

Department of Biochemistry and Biophysics

Pennsylvania State University

University Park, PA 16802

September 1977 to May 1988

Supervisor: Dr. Wallace Snipes

**Project:**

- I. Inactivation of lipid-containing viruses through physical perturbation of membranes.

**Gary R. Matyas**

**Curriculum Vitae (continued)**

**Teaching**

**Instructor**

Introductory Microbiology Laboratory  
Department of Biological Sciences  
Purdue University  
West Lafayette, IN 47907  
January 1981 to May 1981  
Supervisor: Dr. David Filmer

**Teaching Assistant**

Introductory Microbiology Laboratory  
Department of Biological Sciences  
Purdue University  
West Lafayette, IN 47907  
August 1979 to May 1980, August 1978 to May 1979  
Supervisors: Dr. Allen Konopka and Dr. David Filmer

**AWARDS:**

**Special Purdue Fellowship**

Purdue University  
West Lafayette, IN 47907  
August 1980 to December 1980; \$2,000

**Marion County Cancer Society (Little Red Door) Fellowship**

1801 North Meridian Street, Indianapolis, IN 46202  
"A New Serodiagnostic Parameter for Early Detection of Cancer  
That May Distinguish Localized and Disseminated Disease"  
January 1982 to December 1982; \$12,498

**Milheim Foundation for Cancer Research**

Colorado National Bank  
Seventeenth Street at Champs  
Denver, CO 80202  
"The Biochemical Basis for Metastasis"  
July 1982 to June 1983; \$11,421

**Gary R. Matyas**

**Curriculum Vitae (continued)**

**David Ross Fellowship**

**Purdue University**

**West Lafayette, IN 47907**

**"Protein Kinase Modulations as Early Events of Tumorigenic Progression"**

**August 1983 to July 1985; \$13,200**

**MEMBERSHIPS:**

**American Association for the Advancement of Science  
1978 to present**

**American Society for Biochemistry and Molecular Biology  
1987 to present**

**American Society of Microbiology  
1996 to present**

## BIBLIOGRAPHY

Gary R. Matyas

### PUBLICATIONS:

1. Matyas, G. R., Werderitsh, D. A., Morre, D. M. and Morre, D. J.: Comparison of Metastatic and Non-metastatic Transplantable Tumors from the Jaw Region of the Rat. Proceedings of the Indiana Academy of Science (1981) 91, 161-173.
2. Matyas, G. R. and Morre, D. J.: Coupling of Uridine-5'-Diphosphate (UDP) Formation and Nicotinamide Adenine Dinucleotide (NADH) Reduction for Cytochemical Localization of Glycosyltransferases. Journal of Histochemistry and Cytochemistry (1983) 31, 1175-1182.
3. Matyas, G. R. and Morre, D. J.: Isolation of Purified Membranes and Membranous Cell Components for Receptor Studies. Investigations of Membrane-Located Receptors (Methodological Surveys (B): Biochemistry Vol. 12, Eds. Reid, E., Cook, G. M. W. and Morré, D. J., Plenum Publishing Corp., New York, NY (1984) pp. 111-118.
4. Morre, D. J., Creek, K. E., Matyas, G. R., Minnifield, N., Sun, I., Baudoin, P., Morré, D. M. and Crane, F. L.: Free-flow Electrophoresis for Subfractionation of Rat Liver Golgi Apparatus. Biotechniques (1984) 2, 224-233.
5. Morre, D. J., Matyas, G. R. and Mollenhauer, H. H.: Dictyosome-Like Structures from Guinea-Pig Testes Lack Galactosyltransferase, a Golgi Apparatus Marker. Cell Tissue Research (1985) 240, 35-40.
6. Matyas, G. R., Walter-Doelling, V. P., Ferroli, C., Pennington, K. R., Pikaard, D. and Morre, D. J.: Glycolipid Antigens: Potential in Cancer Detection. Investigation and Exploitation of Antibody Combining Sites (Methodological Surveys (B): Biochemistry Vol. 14, Eds. Reid, E., Cook, G. M. W. and Morre, D. J., Plenum Publishing Corp., New York, NY (1986) pp. 323-332.
7. Matyas, G. R., Evers, D. C., Radinsky, R. and Morre, D. J.: Fibronectin Binding to Gangliosides and Rat Liver Plasma Membranes. Experimental Cell Research (1986) 162, 296-318.
8. Sallay, S. I., Prorise, W. W., Morre, D. J. and Matyas, G. R.: Ganglioside and PCA Sialic Acid Serum Levels in Cancer. The Cancer Journal (1986) 1, 124-129.
9. Nakane, N., Morgan, D. E., Matyas, G. R., Morre, D. M. and Morré, D. J.: Blood Coagulation Abnormalities in Fischer Strain Rats Bearing Tumors. Life Sciences (1987) 40, 2523-2529.



Gary R. Matyas

Bibliography (cont.)

10. Matyas, G. R., Aaronson, S. A., Brady, R. O. and Fishman, P. H.: Alteration of Glycolipids in ras Transfected NIH 3T3 Cells. Proceedings of the National Academy of Science USA (1987) 84, 6065-6068.
11. Matyas, G. R. and Morre, D. J.: Subcellular Distribution and Biosynthesis of Rat Liver Gangliosides. Biochimica et Biophysica Acta (1987) 921, 599-614.
12. Spiegel, S., Matyas, G. R., Cheng, L. and Sacktor, B.: Asymmetric Distribution of Gangliosides in Renal Brush Border and Basolateral Membranes. Biochimica et Biophysica Acta (1988) 938, 270-278.
13. Matyas, G. R., and Fishman, P. H.: Lipid Signalling Pathways in Normal and Ras-Transfected NIH/3T3 Cells. Cellular Signalling (1989) 1, 395-404.
14. Buckley, N. E., Matyas, G. R. and Spiegel, S.: The Bimodal Growth Response of Swiss 3T3 Cells to the B Subunit of Cholera Toxin Is Independent of the Density of Its Receptor, Ganglioside, GM1. Experimental Cell Research (1990) 189, 13-21.
15. Wassef, N. M., Matyas, G. R. and Alving, C. R.: Complement-Dependent Phagocytosis of Liposomes By Macrophages: Suppressive Effects of "Stealth Lipids." Biochemical and Biophysical Research Communications (1991) 176, 866-874.
16. Carl R. Alving, Glenn M. Swartz, Nabila M. Wassef, Edward E. Herderick, Remu Virmani, Frank D. Kolodgie, Gary R. Matyas, Jorge L. Ribas, Julie R. Kenner, and J. Frederick Cornhill. Vaccination Against Cholesterol: Immunologic Modulation of Diet-Induced Hypercholesterolemia and Atherosclerosis. In Atherosclerosis X. Eds. F. P. Woodford, J. Dvignon and A. Sniderman, Excerpta Medica International Congress Series, Elsevier Science, Amsterdam, (1995) pp 944-948.
17. Carl R. Alving, Glenn M. Swartz, Nabila M. Wassef, Edward E. Herderick, Remu Virmani, Frank D. Kolodgie, Gary R. Matyas, Jorge L. Ribas, Julie R. Kenner, and J. Frederick Cornhill. Prospects for an Anti-Cholesterol Vaccine. Clinical Immunotherapy (1995) 3, 409-414.
18. Gregory M. Glenn, Mangala Rao, Roberta L. Richards, Gary R. Matyas, and Carl A. Alving. Murine IgG Subclass Antibodies to Antigens Incorporated in Liposomes Containing Lipid A. Immunological Letters (1995) 47, 73-78.

**Gary R. Matyas**

**Bibliography (cont.)**

19. Carl R. Alving, Glenn M. Swartz, Nabila M. Wassef, Jorge L. Ribas, Edward E. Herderick, Renu Virmani, Frank D. Kolodgie, Gary R. Matyas, and Julie F. Cornhill. Immunization with Cholesterol-Rich Liposomes Induces Anti-Cholesterol Antibodies and Reduces Diet-Induced Hypercholesterolemia and Plaque Formation. Journal Laboratory and Clinical Medicine (1996) 127:40-49.
20. Gary R. Matyas and Carl R. Alving. Protective Prophylactic Immunity Against Intranasal Ricin Challenge Induced by Liposomal Ricin A Subunit. Vaccine Research (1996) 5:163-172.

**MANUSCRIPTS SUBMITTED:**

1. Gregory M. Glenn, Mangala Rao, Gary R. Matyas, Patricia Walker and Carl R. Alving. Transcutaneous Immunization Using Bacterial ADP-Ribosylating Exotoxin. Science (submitted).
2. Mangala Rao, Gary R. Matyas, Franziska Grieder, Kevin Anderson, Peter B. Jahrling, and Carl R. Alving. Cytotoxic T Lymphocytes to Ebola Zaire Virus Are Induced in Mice by Immunization with Liposomes Containing Lipid A. European Journal of Immunology (submitted).

**ABSTRACTS:**

1. Matyas, G. R. and Werderitsh, D. A.: Comparison and Ganglioside Composition of Metastatic and Non-metastatic Transplantable Tumors from the Jaw Region of the Rat. Federation Proceedings (1981) 40, 1544.
2. Morré, D. J., Hsu, H., Matyas, G. R., Kloppel, T., Classen, P. and Jacobson, W.: Lipid-associated Sialic Acid (LASA) Elevated in Sera of Carcinoma Patients. Proceedings of the American Association of Cancer Research (1981) 22, 22.
3. Morgan, D. E., Morré, D. M., and Matyas, G. R.: Effects of Vitamin A on Various Hematological Parameters of Rats Bearing Tumors. Proceedings of the Indiana Academy of Science (1981) 91, 147.
4. Matyas, G. R., Morré, D. J. and Keenan, T. W.: Distribution of Gangliosides Among Subcellular fractions of Rat Liver. Federation Proceedings (1982) 41, 1170.
5. Jacobson, L. B., Putman, J. E., Matyas, G. R., Morré, D. M. and Morré, D. J.: Gamma-glutamyl Transpeptidase Activity of Transformed Rat Hepatocytes. In Vitro (1982) 18, 285.

Gary R. Matyas

Bibliography (cont.)

6. Morré, D. J., Croza, E. M., Matyas, G. R. and Evers, D. C.: Isolated Membrane Fractions for Probing Membrane-Located Receptors in Biochemical Studies. Eighth International Subcellular Methodology Forum (1982) University of Surrey, England.
7. Matyas, G. R. and Morré, D. J.: Cytochemical Localization of Glycosyl Transferases. Journal of Cell Biology (1982) 95, 510.
8. Matyas, G. R., Evers, D. C., Radinsky, R., and Morré, D. J.: Ganglioside Composition of Rodent Tumors and Fibronectin Binding. Proceeding of the American Association of Cancer Research (1983) 24, 27.
9. Matyas, G. R. and Morré, D. J.: Synthesis and Transport of Gangliosides by Rat Liver. Federation Proceedings (1983) 42, 2019.
10. Morré, D. J., Matyas, G. R., Pikaard, D., Chu, A. and Pennington, K.: Levels of Ganglioside-Bound Sialic Acid Elevated in Sera of Cancer Patients. Proceedings of the American Association for Cancer Research (1984) 25, 157.
11. Morré, D. J., Morré, D. M. and Matyas, G. R.: Golgi Apparatus Subfraction by Free-Flow Electrophoresis Separates Cis and Trans Elements. Federation Proceedings (1984) 43, 1715.
12. Matyas, G. R., Pennington, K. and Morré, D. J.: Levels of Ganglioside-Bound Sialic Acid Elevated in Sera of Cancer Patients. Tenth International Subcellular Forum (1984) University of Surrey, England.
13. Matyas, G. R., Creek, K. E., Morré, D. J. and Morré, D. M.: Evidence from Free-Flow Electrophoresis and from Enzyme Cytochemistry from a Cis to Trans Gradient of Galactosyl- and Sialyltransferase Across the Polarity Axis of Rat Liver Golgi Apparatus. Journal of Cell Biology (1984) 99, 104a.
14. Esposito, R. V., Matyas, G. R., Blyden, S. and Morré, D. J.: Molecular Crosslinking to Determine Near Neighbor Relationships Between Fibronectin and Gangliosides of Rat liver plasma Membranes. Federation Proceedings (1986) 45, 1821.
15. Nowack, D., Navas, P., Matyas, G. R., Morré, D. M. and Morré, D. J.: Characteristics of the Golgi Apparatus of Liver-Like Rat Hepatoma RLT-N. Biological Chemistry, Hoppe-Seyler (1986) 367, 152.
16. Matyas, G. R.: Alteration of Glycolipids in *ras* Transfected NIH3T3 Cells. Journal of Cellular Biochemistry (1987) Supplement 11D, 152.

**Gary R. Matyas****Bibliography (cont.)**

17. Matyas, G. R.: Elevations fo Diacylglycerol in *ras* Transfected NIH/3T3 Cells Are Not From Phosphoinositide Hydrolysis. Journal of Cell Biology (1988) 107, 705a.
18. Buckley, N., Matyas, G. R. and Spiegel, S.: Bimodal Regulation of Cell Growth By Gangliosides. Proceedings of the Xth International Symposium on Glycoconjugates (1989) 290.
19. Matyas, G. R., Wassef, N. M., Wilhelmson, C. I., Johnson, A. J. and Alving, C. R.: Concanavalin A: Cytotoxic Effects on Murine Macrophage P388D Cells and Physiologic Effects of Toxic Doses *in Vivo*. The FASEB Journal (1990) 4, 1989.
20. Wassef, N. M., Matyas, G. R. and Alving, C. R.: Complement-Dependent Phagocytosis of Liposomes by Macrophages: Suppressive Effects of Gangliosides and Phosphatidylinositol. The FASEB Journal (1990) 4, 2141.
21. Buckley, N. E., Matyas, G. R. and Spiegel, S.: Swiss 3T3 Fibroblasts Display a Bimodal Growth Response to the B Subunit of Cholera Toxin Independent of the Concentration of Ganglioside GM1. The FASEB Journal (1990) 4, 1912.
22. Mayorga, M. A., Matyas, G. R., Wilhelmson, C. L., Banks, R. E. and Alving, C. R.: Acute Respiratory Injury Induced By Endotracheally Instilled Snake Venom Phospholipase A<sub>2</sub> (PLA<sub>2</sub>). The FASEB Journal (1991) 5, 1268.
23. Matyas, G. R. and Fishman, P. H.: Endogenous Gangliosides Do Not Modulate But Are Modulated By Cell Growth in Murine 3T3 Cells. The FASEB Journal (1991) 5, 1547.
24. Mayorga, M.A., Matyas, G., Wilhelmsem, C., Banks, R., Alving C.: Production of Monoclonal Antibodies to Phospholipase A2: Potential Antidote for Phospholipase A2 Toxicity. American College of Physicians, Army Regional Meeting, San Francisco, CA 4-8 Nov 92.
25. Matyas, G. R. and Alving, C. R.: Effect of Route of Administration on Generation of Different Isotype Antibodies to Liposome-Encapsulated Ricin Subunits. The FASEB Journal (1994) 8, A681.
26. Matyas, G. R. and Alving, C. R.: Effect of Route of Administration on Generation of Different Isotype Antibodies to Liposome-Encapsulated Ricin Subunits. The FASEB Journal (1994) 8, A1464.
27. G. M. Glenn, M. Rao, R. Owens, G. Matyas, C. R. Alving. Murine IgG Subclass and T-helper Response to Cholera Toxin and Sp866 Incorporated in Liposomes Containing Lipid A. Presented National Meeting of Leukocyte Biology, 20-24 September 94.

**Gary R. Matyas****Bibliography (cont.)**

28. Alving, C. R., Swartz, G. M., Wassef, N. M., Herderick, E. E., Virmani, R., Kolodgie, F. D., Matyas, G. R., Ribas, J. L., Kenner, J. R., and Cornhill, J. F. . Vaccination Against Cholesterol: Immunologic Modulation of Diet-Induced Hypercholesterolemia and Atherosclerosis. X<sup>TH</sup> International Symposium on Atherosclerosis, Montreal, 09-14 October 1994.
29. Matyas, G. R. and Alving, C. R.: Production of Serum IgA and High Titer IgG Antibodies by Intranasal Immunization with Liposome-Encapsulated Ricin Subunits. The FASEB Journal (1995) 9, A216.
30. Matyas, G. R. and Alving, C. R.: Protection Against Ricin by Intranasal Immunization with Liposomes. FASEB Journal (1996) 10:A1190.
31. Harris, D. T., Mastrangelo, M. J., Alving, C., Matyas, G., Hwang, M., Cox, J., Maida, A. and Spitler, L. E.: Active Specific Immunization of Patients with Hormone-Refractory Prostate Cancer Using OncoVax-P<sup>TM</sup>. Proceedings of the American Society of Clinical Oncology (1996) 15, 555.
32. Talmadge, J., Muderhwa, J., Alving, C., Matyas, G., Hwang, M., Maida, A., Spitler, L.: Preclinical Evaluation of OncoVax-P<sup>TM</sup>, a Prostate Cancer Vaccine. Proceedings of the American Association for Cancer Research (1996), 37, 477.
33. Talmadge, J., Muderhwa, J., Alving, C., Matyas, G., Hwang, M., Maida, A. and Spitler, L.: GM-CSF as an Adjuvant with OncoVax-P<sup>TM</sup>, a Prostrate Cancer Vaccine. Proceedings of the American Association for Cancer Research (1997) 38, 400.

# INDUCTION OF CYTOTOXIC T-LYMPHOCYTES TO TUMOR CELLS PRESENTING PROSTATE SPECIFIC ANTIGENS

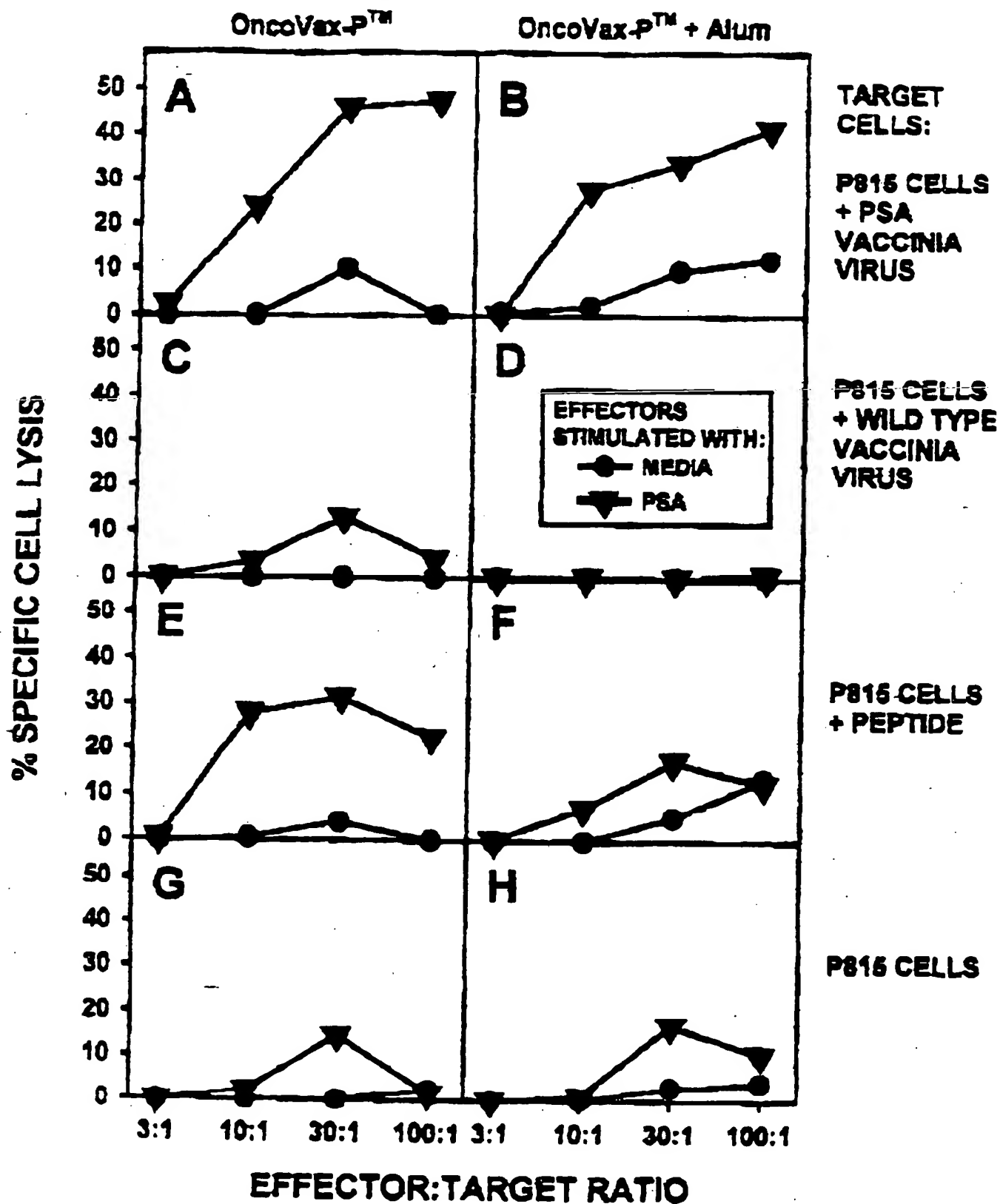


Exhibit B

Matyas

## Induction of Antitumor Response in Mice by Prostate Cancer Vaccine

Gary Maryas, PhD, Mangala Rao, PhD, Jean Muderhwa, PhD, and Carl Alving, MD, Walter Reed Army Institute of Research, Washington, DC

We report that immunization of mice with OncoVax-P<sup>TM</sup> prostate cancer vaccine induces cytotoxic T-lymphocytes which have the capability of killing tumor cells presenting PSA antigens.

Two groups of Balb/c mice (6 mice/group) were immunized with OncoVax-P<sup>TM</sup>. One group was immunized with OncoVax-P<sup>TM</sup> alone and the other group received OncoVax-P<sup>TM</sup> mixed with aluminum hydroxide (alum, Alhydrogel) (200 µg Al<sup>+++</sup>). OncoVax-P<sup>TM</sup> is a liposomal formulation of prostate specific antigen (PSA) with lipid A, as an adjuvant. The PSA dose was 5 µg PSA and 20 µg of lipid A. The mice were immunized at week 0 and 4 weeks after the boost. Three animals per group were euthanized 4 weeks after the boost and the spleens were removed for CTL assay. The cells from each group of spleens were pooled and incubated 5 days with media alone or 2 µg/ml human PSA. Target P815 cells (mouse mastocytoma) were infected with either wild type vaccinia virus (WTvac) or with vaccinia virus transfected with PSA gene and the *E. coli* lac Z gene (PSA<sub>vac</sub>, Therion Biologics Corporation) at an multiplicity of infection of 10. P815 cells were incubated with 10 µg/ml of peptide, CYASGWGSI, which represents a potential murine CTL epitope for PSA. It was identified using the University of Wisconsin Genetics Computer Group sequence analysis finding pasterns computer program. This program was used to compare the amino acid sequence of PSA with the known murine CTL anchor sequences. CYASGWGSI is located at amino acid positions 128-137 of PSA. The P815 targets were incubated for 16 hours with vaccinia viruses or peptide. The targets cells were then labeled with Cr<sup>51</sup> (0.1 µCi/10<sup>6</sup> cells) for one hour. Effector cells were harvested and plated in 96 well U bottom plates. Cr<sup>51</sup> labeled targets were added at various effector:target ratios. Following centrifugation at approximately 50 X g for 5 min, the plates were incubated at 37°C for 5 hr. The radioactivity present in the supernatant was harvested using Skatron wicks and the radioactivity was quantified using a gamma counter.

As shown in the attached figure, murine CTL which specifically lysed PSAvac infected targets (A & B) and targets incubated with peptide (E) were obtained. Approximately, 47% cell lysis was obtained using PSAvac targets. WTvac targets were not lysed (C & D), indicating the CTLs were specific for PSA epitopes. Addition of alum to the vaccine had no effect on the generation of CTL which lysed PSAvac targets (A & B) but it inhibited the induction of CTLs to tumor cells pulsed with peptide.

These results indicate that immunization of mice with the prostate cancer vaccine induces lymphocytes capable of specifically killing tumor cells presenting PSA antigens indicating an antitumor response.



CERTIFICATE OF MAILING BY "FIRST CLASS MAIL"

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to:  
Assistant Commissioner for Patents, Washington, D.C. 20231, on April \_\_, 1998.

Date

Alexandra H. Parsons

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

hand  
delivered  
4/29/98

In the application of:

Lynn E. Spitler et al.

Serial No.: 08/288,057

Filing Date: 10 August 1994

For: PROSTATIC CANCER VACCINE

Examiner: P. Gambel

Group Art Unit: 1816

**DECLARATION OF LYNN E. SPITLER, M.D.  
PURSUANT TO 37 C.F.R § 1.132**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, Lynn E. Spitler, declare as follows:

1. I am a coinventor of the subject matter in the above-referenced application and have been a practicing immunologist for many years. My *curriculum vitae* is of record in this application.

2. I have been and am directing the clinical trials of OncoVax<sup>PTM</sup>, an immunological preparation for effecting an antitumor effect with regard to prostate cancer. OncoVax<sup>PTM</sup> contains recombinant human prostate-specific antigen (rhPSA) formulated into compositions which are designed to behave as adjuvants. In a previous Declaration, executed 26 August 1997, I described the results of four clinical trials which showed promising results for the rhPSA as antigen. Exhibit B to my previously submitted Declaration summarized these results.

3. We have conducted an additional, fifth clinical trial in which the rhPSA was prepared in a liposomal formulation which was then emulsified with mineral oil. A group of 5 patients was treated in this study. They had undergone previous treatment for prostate-derived tumors. The attached Exhibit A contains a summary of the particulars relating to these patients. In 3 of the 5 patients the tumor had metastasized to the bone. The OncoVaxP™ vaccine was administered over the period of August 1997 to November 1997 or September 1997 to December 1997 as shown.

4. As in the previous four trials, several measures of immune response were obtained: skin test reactivity to PSA, production of IgG antibodies, and, most important, proliferation of lymphocytes in response to PSA. As shown in Chart 3 as part of Exhibit A, the 5 patients uniformly gave a dramatic response to OncoVaxP™ in the form of lymphocyte proliferation in response to PSA -- a result associated with the cellular immune system. In all cases, lymphocyte proliferation in response to PSA was dramatically improved after the vaccination protocol.

5. These results are highly indicative of an antitumor effect. The relationship between cell-mediated immunity and control of tumor growth is well documented. Cell-mediated immunity can be measured either by delayed type hypersensitivity (DTH) skin testing or by lymphocyte proliferation responses to the vaccine antigen. These are simply alternative assays for a cellular response. I have attached several examples of this documentation as Exhibits B, C, D and E.

6. In Exhibit B, a paper by Barth, A. *et al. Cancer Research* (1994) 54:3342-3345, discloses that melanoma patients were administered a polyvalent melanoma cell vaccine. The authors noted that overall survival was significantly prolonged in patients with positive DTH reaction and/or an increased cytotoxic T cell activity. For example, Figure 2 in Exhibit B shows a comparison of survival times of patients with DTH  $\geq 6$  mm compared to patients DTH  $< 6$  mm. As shown, the survival time of the responders was dramatically increased ( $p=0.0054$ ). The median survival was 52 months in the DTH-positive group as compared to 22 months in the DTH-negative group.

7. As shown in Exhibit C, an abstract of an article by Bystryń, J.C. *et al. Cancer* (1992) 69:1157-1164, the purpose of this study was to determine whether there was a relationship between induction of DTH in response to vaccination with a melanoma vaccine and

disease recurrence. Melanoma patients were administered a partially purified polyvalent melanoma antigen vaccine. The median disease-free survival of patients was correlated with their response to DTH. A statistically significant relationship was found. The median disease-free survival of patients with a strong DTH response was 4.7 years longer than that of those who did not respond.

8. In Exhibit D, an abstract of a paper by Berd, D. *et al. J Clin Oncol* (1997) 15:2359-2370, melanoma patients were administered autologous whole cells modified with the hapten dinitrophenyl (DNP). The authors noted that the development of a positive DTH response to the autologous melanoma cells which were unmodified with DNP was correlated with a significantly longer five-year survival.

9. Finally, Exhibit E is an abstract of an article by McCune, C.S. *et al. Cancer Immunol Immunother* (1990) 32:62-66. Patients with metastatic renal cell carcinoma were vaccinated with autologous tumor cells and skin-tested for an immunological response. The authors concludes that the survival times of the skin-test-positive group were significantly superior to those of the skin-test-negative group.

10. The articles cited are only four of many which show that a response by the cellular immune system to tumor-associated antigens is correlated with antitumor effect and clinical benefit as demonstrated by increased disease-free survival and/or survival time. Generally, it is recognized that this correlation is strong. Much of the difficulty in obtaining an immune response lies in the difficulty of assuring a uniform, potent composition to administer, a problem not encountered with rhPSA.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Wash DC, ~~California~~ on April 29 1998, by

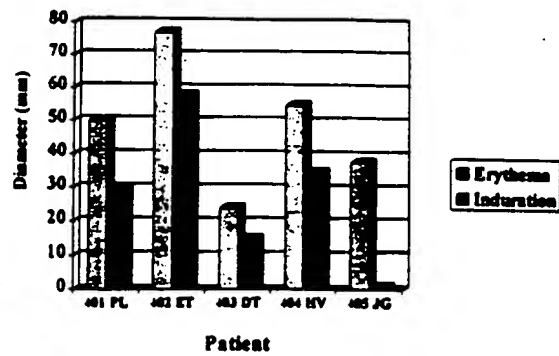
  
\_\_\_\_\_  
Lynn E. Spitler, M.D.

**Clinical Trial #5**  
**Administration of OncoVax-PM Emulsion**  
**February 6, 1998**

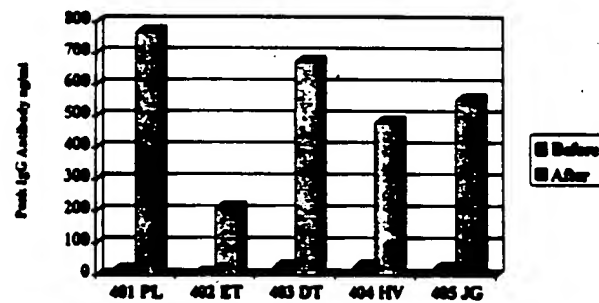
Patient	Age	Prior Treatment	Sites	Vaccine	PSA Pre	PSA D 90	Clin Resp	PSA Last	Post	Last FU	Death
1. PL (#401)	67	Biopsy Orchiectomy Hormonal	Prostate Bone	8/97 - 11/97 1/98	0.98	0.31	S	<0.05 (1/98)	Boost	1/98	
2. ET (#402)	70	Biopsy Hormonal	Prostate Bone	8/97 - 11/97 1/98	46.3	525.80	Improved Bone Scan*	1622.40 (1/98)	Boost	1/98	
3. DT (#403)	74	Biopsy Radiation Hormonal	Prostate	8/97 - 11/97 1/98	9.91	13.55	S	6.51 (1/98)	Boost	1/98	
4. HV (#404)	70	Biopsy Radiation Hormonal	Prostate Bone	9/97 - 12/97	18.13	51.46	S		Boost	12/97	
5. JG (#405)	75	Biopsy Radiation	Prostate	9/97 - 12/97	35.40	39.91	S			12/97	

\* Alkaline phosphatase 1200 → 300

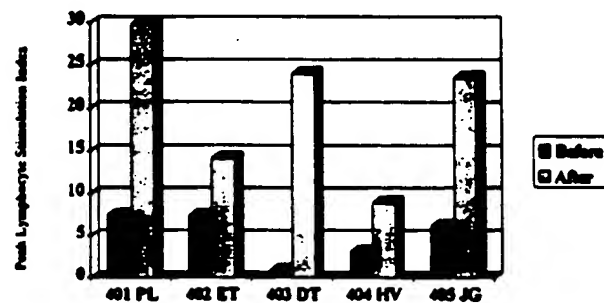
### OncoVax-P Emulsion Skin Test Reactivity to PSA



### OncoVax-P Emulsion IgG Antibody Reactivity to PSA



### OncoVax-P Emulsion Lymphocyte Proliferation to PSA



# Polyvalent Melanoma Cell Vaccine Induces Delayed-Type Hypersensitivity and *in Vitro* Cellular Immune Response<sup>1</sup>

Andreas Barth, Dave S. B. Hoon, Leland J. Foshag, J. Anne Nizze, Estela Famatiga, Edward Okun, and Donald L. Morton<sup>2</sup>

John Wayne Cancer Institute at Saint John's Hospital and Health Center, Santa Monica, California 90404

## Abstract

Patients with melanoma metastatic to distant sites or at high risk for recurrent melanoma have been treated with a polyvalent melanoma cell vaccine (MCV) in phase II protocols. We assessed *in vivo* and *in vitro* cell-mediated responses to MCV in 163 patients who had undergone surgical resection of American Joint Committee on Cancer stage III melanoma. During the first 4 months of vaccine immunotherapy, 135 patients (83%) responded by developing a positive delayed-type hypersensitivity reaction  $\geq 6$  mm to MCV. In a mixed lymphocyte tumor cell reaction using peripheral blood lymphocytes, 35 of 42 patients (83%) showed a recall proliferative response to one or more of the three cell lines of MCV. There was a significant correlation between delayed-type hypersensitivity reaction and mixed lymphocyte tumor cell reaction ( $P = 0.013$ ). After 4 months of MCV therapy, 8 of 11 patients had an increased mixed lymphocyte tumor cell reaction to autologous melanoma cells. During the first 4 months of vaccine therapy, 16 of 33 patients developed more than a 50% increase in cytotoxic T-cell activity against one of the cell lines of MCV. Overall survival was significantly prolonged in patients with a positive delayed-type hypersensitivity reaction ( $P = 0.0054$ ) and/or increased cytotoxic T-cell activity ( $P = 0.02$ ). These findings suggest that MCV induces specific T-cell responses which are correlated with clinical course; the data also suggest that some of these responses are directed against autologous melanomas and may play a major role in controlling the progression of melanoma.

## Introduction

Melanoma patients with regional soft tissue or lymph node metastases (AJCC<sup>3</sup> stage III disease) have a high risk of recurrence and a 10-year survival rate of 15–40%, depending on the extent of nodal involvement (1). In the absence of effective adjuvant chemotherapy protocols for melanoma, many investigators are attempting to induce or augment specific T-cell responses that may control disease progression (2–8). Our recent phase II study showed that patients whose advanced melanoma was treated with repeated intradermal injections of polyvalent allogeneic MCV had a significantly prolonged overall survival compared to historic controls (5). The present report analyzes the magnitude of the T-cell response to MCV in AJCC stage III melanoma patients.

## Materials and Methods

**Patients.** We developed MCV in 1984 and initiated our phase II trial of MCV immunotherapy on September 25, 1984. In the present report, the study

population of 163 patients treated between January 1, 1985, and July 1, 1989, represented all patients who had undergone surgical resection of AJCC stage III melanoma, and were followed at least 3.5 years after beginning postoperative MCV immunotherapy. There were 99 males and 64 females; their median age was 41 years (range, 16–79). The majority (63%) of the primary lesions were on the head and neck or the trunk; the remaining 37% were on the extremities or of unknown primary site. Only 33% of the primaries were thin lesions ( $<1.5$  mm). Before surgery, 21% of the patients had in-transit metastases and 79% had lymph node metastases (24% had 1 positive lymph node, 30% had 2–4 positive nodes, and 25% had  $\geq 5$  positive nodes). All patients were clinically free of disease after surgery, as determined by physical examination and radiographic imaging of brain, chest, abdomen, and pelvis. All had a life expectancy of more than 6 months and normal blood count, liver enzymes, and creatinine. None of the female patients was pregnant. No patient had received immuno-, chemo-, or radiation therapy within the past 30 days. Written consent for MCV immunotherapy was obtained from all patients, and the MCV protocol was approved by the Human Subjects Protection Committees of the John Wayne Cancer Institute and Saint John's Hospital and Health Center, and the UCLA Jonsson Comprehensive Cancer Center.

**MCV Cell Lines and Preparation.** MCV comprises three well-characterized allogeneic melanoma cell lines (M10, M24, and M101). These cell lines were established in our laboratory and selected for their content of immunogenic MAA (5). Their HLA class I types are as follows: M10 (A24,33; B35,38), M24 (A11,33; B35,62), and M101 (A2,29; B44). Their surface expression of HLA-DR antigen is  $<2\%$ .

The preparation of MCV is described elsewhere (5). Briefly, cells from each line are grown in serum-free medium, harvested, and pooled ( $8 \times 10^6$  cells/line;  $24 \times 10^6$  total cells per vaccine treatment). Individual batches of MCV are analyzed for antigen expression and screened for infectious disease contaminants. MCV is irradiated at 100–150 Gy and then cryopreserved. Immediately before administration, the vaccine is thawed and washed 3 times in sterile physiological phosphate-buffered saline.

**MCV Administration.** MCV therapy was initiated within 3 months after surgical removal of regional lymph node or soft tissue disease (9), using a previously described protocol (5). The vaccine was injected intradermally in axillary and inguinal regions every 2 weeks  $\times 3$ , and then monthly for a year. After 1 year the interval was increased to every 3 months  $\times 4$ , and then every 6 months. For the first two treatments MCV was mixed with the Tice strain of *Bacillus Calmette-Guerin* (Organon Teknika Corp., Durham, NC) ( $8 \times 10^6$  organisms). Some patients received immunomodulators such as cimetidine (SmithKline, Philadelphia, PA), indomethacin (Lederle, Wayne, NJ), or cyclophosphamide (Mead Johnson, Princeton, NJ) (5). Clinical and laboratory evaluations were performed at each vaccine administration; chest X-rays were repeated every 2 months for the first year and then with each vaccine administration.

**DTH.** Equal amounts of each MCV cell line were pooled to a total dose of  $2.4 \times 10^6$  cells and administered intradermally at a remote site on the forearm. DTH was defined as the average diameter of induration after 48 h. A positive response was  $\geq 6$  mm of induration, independent of accompanying erythema. The greatest DTH during the first 4 months was used for survival analysis. Since autologous cell lines were not available during the first 16 weeks of MCV therapy, autologous DTH could not be performed.

**MLTR.** Cryopreserved PBL from weeks 0, 4, and 16 of MCV immunotherapy were available for testing from 42 AJCC stage III patients. These PBL were simultaneously thawed, washed, and resuspended in AIM-V culture

Received 2/21/94; accepted 5/18/94.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup>Supported by Grant CA 12582, National Cancer Institute, Bethesda, MD; the Cancer League of Bern, Switzerland; and the Joyce and Ben Eisenberg Foundation.

<sup>2</sup>To whom requests for reprints should be addressed, at John Wayne Cancer Institute, 2200 Santa Monica Blvd., Santa Monica, CA 90404.

<sup>3</sup>The abbreviations used are: AJCC, American Joint Committee on Cancer; MAA, melanoma-associated antigens; MCV, melanoma cell vaccine; DTH, delayed-type hypersensitivity reaction; PBL, peripheral blood lymphocytes; MLTR, mixed lymphocyte tumor cell reaction; PHA, phytohemagglutinin; CTL, cytotoxic T-lymphocytes; LU, lytic units.

medium (GIBCO, Grand Island, NY) containing 10% human AB heat-inactivated serum (Irvine Scientific, Santa Ana, CA), and stimulated at a 5:1 ratio (PBL:melanoma) with each MCV line. In eight cases, a melanoma cell line developed from the patient's biopsy specimen was available for autologous MLTR (10). Autologous MLTR was also performed by using melanoma cell lines developed from biopsy specimens of three additional patients receiving MCV after surgical resection of distant metastases (AJCC stage IV disease).

The MLTR assay was performed as described elsewhere (11). Briefly, PBL were cocultured in triplicate 96-well microplates containing 200  $\mu$ l of culture medium supplemented with 20 units/ml of recombinant interleukin 2 (Cetus, Emeryville, CA). Cells were then incubated for 6 days at 37°C. Respective control cultures of PBL were assayed in medium alone and with PHA (Burroughs-Wellcome, Triangle Park, NC) at a suboptimal concentration of 0.1  $\mu$ g/ml. During the last 18 h of the 6-day assay, cells were pulsed with [ $^3$ H]thymidine (New England Nuclear, Boston, MA) and harvested. Data were analyzed as mean cpm for triplicate measurements (SD < 15%) at each time point.

**Correlation of MLTR with DTH.** Since DTH represents a combined reaction against all three MCV cell lines, MLTR responses to each line were combined to create a MLTR index. To determine this index, the cpm of each cell line at week 0, 4, or 16 was divided by its cpm at week 0. The MLTR index for a specific week was the sum of these values; thus, the MLTR index was always 3 at week 0. The correlation between DTH and MLTR index was determined by linear regression analysis.

**Cytotoxic T-Cells.** Cryopreserved PBL obtained before treatment (week 0) and after 4 and 16 weeks of MCV therapy were simultaneously thawed, washed, and grown for 4 days in AIM-V culture medium containing 10% human AB heat-inactivated serum, 10 units/ml interleukin 2, and 0.1  $\mu$ g/ml PHA. CTL-mediated lysis of MCV cell lines was assessed in a standard 4-h  $^{51}$ Cr-release assay using three effector:tumor target cell ratios (11). Results were expressed in LU according to the formula described by Pross and Maroun (12). One lytic unit was defined as the number of effector cells required to lyse 33% (LU<sub>33</sub>) of  $5 \times 10^3$  target cells. CTL assays were performed early during MCV treatment, without further knowledge of the patient's clinical status. Analysis of representative stimulated lymphocyte subpopulations by flow cytometry with specific T-cell monoclonal antibodies revealed 88% CD3+ and 50% CD8+.

**Survival Analysis.** The overall survival curves were estimated by the Kaplan-Meier method. The log-rank test was used to determine survival differences among patient subgroups defined by DTH or cytotoxicity. All tests were two-sided. Survival time was defined as the interval between the initiation of MCV therapy and the patient's death.

## Results

**DTH.** The mean DTH of the 163 patients increased significantly from a base line of  $3.2 \pm 0.4$  mm (mean  $\pm$  SEM) pretreatment to a maximum of  $13.5 \pm 1.1$  mm at week 4 ( $P < 0.01$ ), and dropped slightly to about 10 mm for the following 3 months. One hundred thirty-five patients (83%) had a maximum DTH  $\geq 6$  mm within 4 months of beginning MCV therapy, while the remaining 28 patients (17%) exhibited no DTH response to MCV ( $< 6$  mm). Maximum DTH was  $15.3 \pm 1.2$  mm for responders (week 4; Fig. 1A) and  $3.6 \pm 1.1$  mm for nonresponders (week 16; Fig. 1B). The distribution of positive lymph nodes was similar between the two groups ( $P = 0.8$ ), as was the thickness of the primary lesion. After a median follow-up of 60 months (range, 43–93), patients with a positive DTH had a median overall survival of 52 months, compared to only 22 months in patients with no DTH response ( $P = 0.0054$ ) (Fig. 2). Patients with a DTH  $\geq 6$  mm and involvement of 1, 2–4, or  $\geq 5$  nodes had 5-year survival rates of 59, 46, and 34%, respectively.

**Autologous MLTR.** Autologous tumor cell lines were available for 11 patients. Week 16 was selected as the evaluation point based upon the number of vaccinations ( $n = 5$ ) and the plateau phase for DTH. After 16 weeks of active specific immunotherapy with MCV, 5 of the 11 patients demonstrated more than a 3-fold increase in proliferative response to their own melanoma (Fig. 3); three other patients showed more than a 1.5-fold increase. Of the three remaining patients

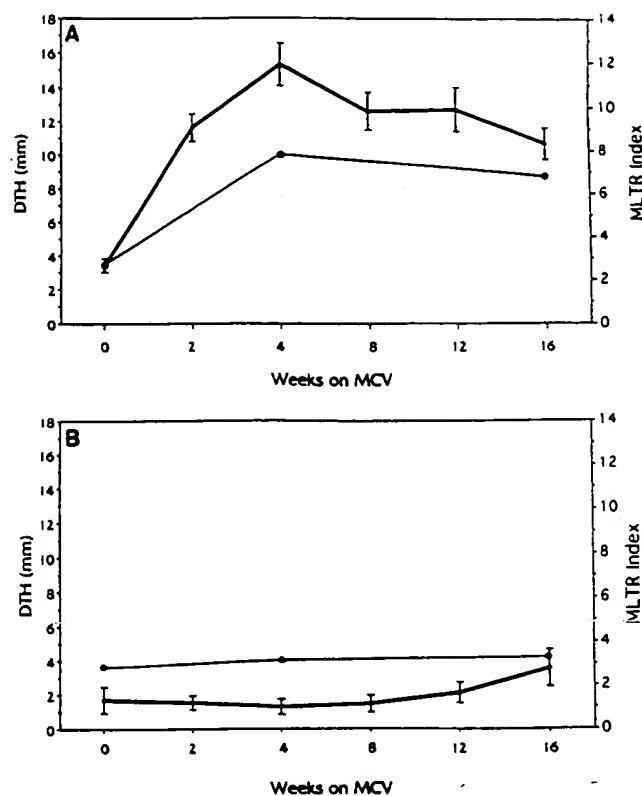


Fig. 1. *In vivo* and *in vitro* T-cell responses to MCV. —, DTH (mean  $\pm$  SEM); —, MLTR index. A, DTH of 135 patients with a maximum DTH  $\geq 6$  mm and MLTR index of 35 responders. B, DTH of 28 patients with maximum DTH  $< 6$  mm and MLTR index of 7 nonresponders. Maximum MLTR was 7.8 for responders and 3.3 for nonresponders. The magnitude of the response was independent of the number of positive lymph nodes ( $P = 0.8$ ).

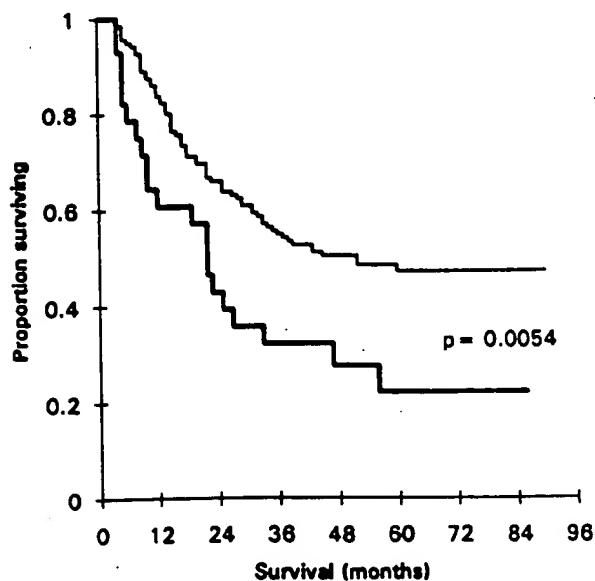


Fig. 2. Overall survival of 163 AJCC stage III melanoma patients undergoing MCV adjuvant immunotherapy initiated within 3 months after surgical removal of regional lymph nodes or soft-tissue disease. After a median follow-up of 60 months, the 135 patients with a DTH  $\geq 6$  mm (—) had a significantly longer overall survival than the 28 patients with DTH  $< 6$  mm (---); median, 52 versus 22 months ( $P = 0.0054$ ).

with no increase, two had AJCC stage IV melanoma. The eight patients with an enhanced response also showed increased reactivity to at least one of the cell lines of MCV. The overall MLTR response of the 11 patients was  $28,100 \pm 8,500$  cpm at week 0, significantly

increasing to  $50,000 \pm 12,600$  cpm at week 16 ( $P < 0.01$ ). Stimulation of PBL with interleukin 2 alone (20 units/ml) produced a response in the 11 patients of  $27,300 \pm 8,200$  cpm at week 0, and  $32,700 \pm 10,100$  cpm at week 16. Similarly, stimulation with PHA alone (0.1  $\mu\text{g}/\text{ml}$ ) produced a response in the 11 patients of  $142,500 \pm 44,900$  cpm at week 0, and  $165,700 \pm 52,400$  cpm at week 16. Responses produced by stimulating PBL with either PHA alone or interleukin 2 alone failed to reach statistical significance between weeks 0 and 16.

**Correlation between DTH and MLTR.** PBL from weeks 0, 4, and 16 were available for 42 of the 163 patients. In 35 of 42 patients (83%), MLTR demonstrated a recall proliferative T-cell response to one or more of the three cell lines of MCV. The MLTR index of the 35 responders peaked at week 4 and then decreased slightly to a plateau (Fig. 1A); the 7 nonresponders demonstrated no change in MLTR index during the observed period (Fig. 1B). Linear regression analysis of data for the 42 patients confirmed a significant correlation ( $P = 0.013$ ) between DTH and MLTR index at weeks 4 and 16.

**Cytotoxic T-Cells.** During the first 16 weeks, CTL-mediated lysis of one or more MCV cell lines increased in 16 of 33 patients by more than 50% above pre-MCV values, to at least  $5 \text{ LU}_{33}/10^6$  effector cells (range, 5–51). CTL activity was HLA class I restricted, as demonstrated by blocking with W6/32 (anti-HLA class I) monoclonal antibody, using respective controls. Individual patients showed no significant changes (weeks 0–16) in natural killer-type activity (K562 cell killing). Fourteen of the 16 responders (88%) shared HLA-A alleles with MCV cell lines (13). Responders demonstrated a significant increase in cytotoxicity against HLA-A-matched targets from week 0 to weeks 4 and 16 ( $P < 0.01$ ), whereas cytotoxicity of nonresponders against HLA-A-matched targets decreased slightly (Fig. 4). As demonstrated in our earlier studies (10, 11), a parallel increase in cytotoxicity against HLA-A-matched melanoma and autologous melanoma cells could be observed (Fig. 5). The overall survival of patients who had an increase in CTL activity after MCV treatment was significantly longer than that of nonresponders ( $P = 0.02$ ), in spite of an approximately equal balance of prognostic factors (Fig. 6).

## Discussion

This brief report describes the induction and prognostic significance of *in vivo* and *in vitro* T-cell immunity in AJCC stage III melanoma patients receiving adjuvant MCV immunotherapy. Of 163

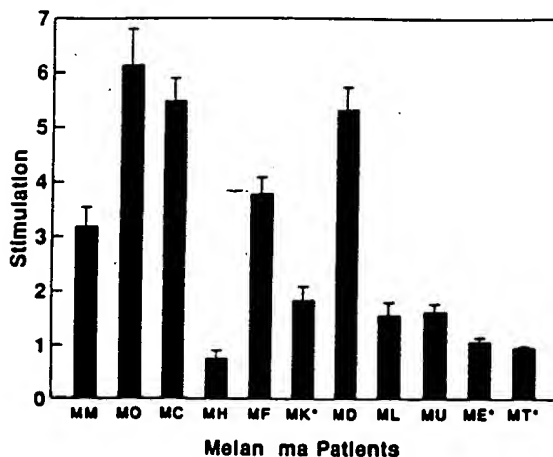


Fig. 3. Autologous MLTR (mean  $\pm$  SD) of 11 patients from whose tumor a cell line was established. Results are expressed as ratios (mean cpm week 16/mean cpm week 0). By week 16 of MCV therapy, the recall proliferative response to autologous tumor had increased more than 3-fold in five patients and more than 1.5-fold in three patients. \*, AJCC stage IV melanoma patient.

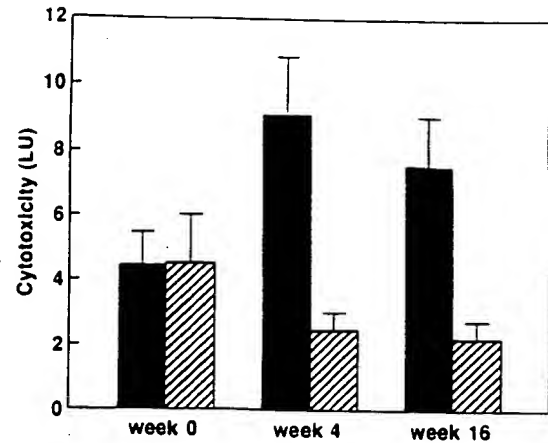


Fig. 4. Cytotoxicity of PBL (mean  $\pm$  SEM) from responders (■,  $n = 14$ ) and nonresponders (▨,  $n = 11$ ) against allogeneic HLA-A-matched melanoma targets. Responders demonstrated a significant increase in cytotoxicity against HLA-A-matched melanoma from week 0 to weeks 4 and 16 ( $P < 0.01$ ), whereas cytotoxicity of nonresponders against HLA-A-matched targets decreased slightly.

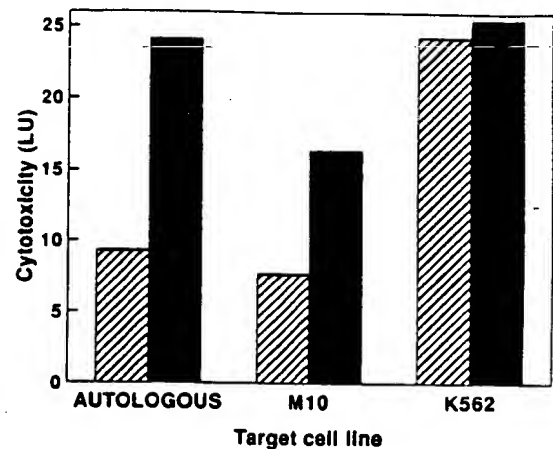


Fig. 5. Cytotoxicity of PBL (A24, 32; B7, 44) against autologous melanoma, allogeneic HLA-A-matched melanoma (M10), and K562. From week 0 (▨) to week 4 (■) there was a parallel increase in cytotoxicity against allogeneic HLA-A-matched and autologous melanoma; there was no change in cytotoxicity against K562.

patients, 135 (83%) developed a DTH  $\geq 6$  mm following vaccine treatment. A similar degree of sensitization was demonstrated by MLTR *in vitro*. Positive DTH to MCV correlated with improved survival; by contrast, in a separate study of 148 AJCC stage IV melanoma patients (14) we were unable to demonstrate a correlation between survival and DTH to common recall antigens (mumps, *Candida albicans*, and purified protein derivative). A significant association between survival and DTH to a tumor vaccine has also been reported by Berd *et al.* (7) and Bystryin *et al.* (6) for high-risk melanoma patients, and by McCune *et al.* (15) for patients receiving active specific immunotherapy against metastatic renal cancer. Bloemen *et al.* (16) recently noted a positive correlation between DTH and *in vitro* proliferative T-cell responses to a mixture of colon tumor-associated antigens. These studies suggest that strong DTH responses during cancer vaccine therapy indicate successful activation of cell-mediated immunity.

MCV may augment T-cell responses in several ways. The enhanced T-cell response to autologous melanoma cells after MCV immunotherapy may result from direct recognition by host T-cells of MAA presented by shared or cross-reactive HLA molecules on MCV lines, as demonstrated *in vitro* (10, 13). Alternatively, activation may occur through antigen processing and presentation of MCV's MAA to host



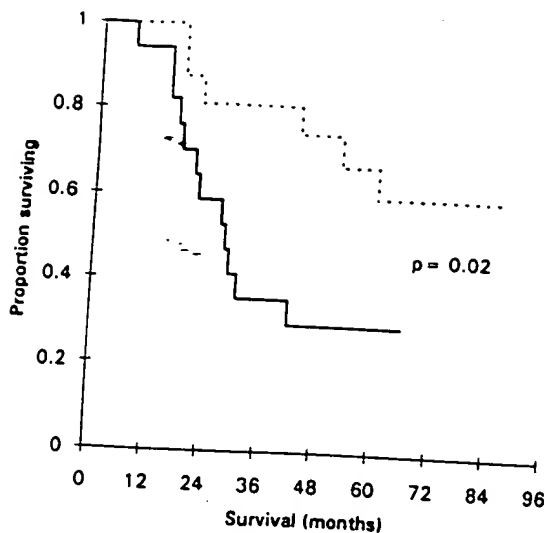


Fig. 6. Overall survival of 33 AJCC stage III melanoma patients undergoing MCV adjuvant immunotherapy initiated within 3 months after surgical removal of regional lymph nodes. After a median follow-up of 61 months, the 16 patients with more than a 50% increase in CTL activity (---) had a significantly longer overall survival (median, >60 months) than the 17 patients with no increase in CTL activity (median, 28 months) (—);  $P = 0.02$ .

T-cells by antigen-presenting cells. The allogeneic HLA antigens on the melanoma cells may stimulate alloreactive T-cells that migrate to the intradermal site of MCV injection; this induces cytokine release and attracts antigen-presenting cells in the microenvironment, which can present common MAA from vaccine cells to MAA-specific T-cells (5, 11, 17–19). This concept was recently suggested by a clinical study showing that *in vivo* transfection of the gene for an allogeneic HLA class I antigen into a patient's melanoma induced specific systemic T-cell immunity (8).

Approximately 20% of our patients did not show a T-cell response to MCV. This may reflect T-cell anergy due to continual exposure of T-cells to MAA and/or cytokine(s) (20). Alternatively, the T-cell response may have been suppressed by factors such as prostaglandins and/or suppressor T-cells (21). Specific biological modifiers administered with MCV may enhance T-cell immune responses; there is evidence that suppressor cells can be inhibited by low doses of cyclophosphamide and indomethacin, thereby up-regulating effector or helper T-cell responses (5, 21, 22).

Augmentation of the T-cell effector response to common MAA may be an important factor in the control of melanoma. In our study, enhanced CTL activity against one or more of the lines of MCV correlated with better survival. In general, CTL kill cancer cells through recognition of MAA as short peptide sequences within HLA molecules expressed on the surface of the cancer cell (23). We and others have demonstrated that CTL can kill allogeneic melanomas expressing the HLA-A antigens of CTL (11, 17, 18). We have also shown that CTL induced by sensitization with allogeneic melanoma cells expressing HLA-A antigens shared by the CTL can recognize and kill autologous melanoma cells (10). These *in vitro* studies suggest recognition of common MAA and support the potential *in vivo* mechanism of MCV cell recognition by MAA-specific T-cells. A defined polyvalent whole-cell MCV immunizes patients with multiple common MAA, thereby inducing an antigen-specific immune response that is effective against different melanoma lesions from the same patient or from different patients. Theoretically, this appears to be one of the most practical approaches to the problem of inducing active specific immunotherapy against tumors that are antigenically heterogeneous. We are currently attempting to characterize MCV antigens that are recognized by T-cells.

## Acknowledgments

We thank Cindy Chang for biostatistical analysis of the data, and the clinical staff of the John Wayne Cancer Clinic.

## References

- Balch, C. M., Soong, S.-J., Shaw, H. M., Urist, M. M., and McCarthy, W. H. An analysis of prognostic factors in 8500 patients with cutaneous melanoma. In: C. M. Balch, A. N. Houghton, G. W. Milton, A. J. Sober, and S.-J. Soong (eds.), *Cutaneous Melanoma*, pp. 165–199. Philadelphia: J. B. Lippincott, 1992.
- Oettgen, H. F., Livingston, P. O., and Old, L. J. Immunotherapy by active specific immunization: clinical applications: melanoma. In: V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), *Biologic Therapy of Cancer*, pp. 682–701. Philadelphia: J. B. Lippincott, 1991.
- Mitchell, M. S., Harel, W., and Groshen, S. Association of HLA phenotype with response to active specific immunotherapy of melanoma. *J. Clin. Oncol.*, 10: 1158–1164, 1992.
- Wallack, M. K., and Sivanandham, M. Clinical trials with VMO for melanoma. *Ann. NY Acad. Sci.*, 690: 178–189, 1993.
- Morton, D. L., Foshag, L. J., Hoon, D. S. B., Nizze, J. A., Famatiga, E., Wanek, L. A., Chang, C., Davtyan, D. G., Gupta, R. K., Elashoff, R., and Irie, R. F. Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine. *Ann. Surg.*, 216: 463–482, 1992.
- Bystryn, J. C., Oratz, R., Roses, D., Harris, M., Henn, M., and Lew, R. Relationship between immune response to melanoma vaccine immunization and clinical outcome in stage II malignant melanoma. *Cancer (Phila.)*, 69: 1157–1164, 1992.
- Berd, D., Maguire, H. C., McCue, P., and Mastrangelo, M. J. Treatment of metastatic melanoma with an autologous tumor-cell vaccine: clinical and immunological results in 64 patients. *J. Clin. Oncol.*, 8: 1858–1867, 1990.
- Nabel, G. J., Nabel, E. G., Yang, Z.-Y., Fox, B. A., Plautz, G. E., Gao, X., Huang, L., Shu, S., Gordon, D., and Chang, A. E. Direct gene transfer with DNA-liposome complexes in melanoma: expression, biologic activity, and lack of toxicity in humans. *Proc. Natl. Acad. Sci. USA*, 90: 11307–11311, 1993.
- Morton, D. L., Wanek, L. A., Nizze, J. A., Elashoff, R. M., and Wong, J. H. Improved long-term survival after lymphadenectomy of melanoma metastatic to regional lymph nodes. *Ann. Surg.*, 214: 491–501, 1991.
- Hayashi, Y., Hoon, D. S. B., Park, M. S., Terasaki, P. I., Foshag, L. J., and Morton, D. L. Induction of CD4+ cytotoxic T cells by sensitization with allogeneic melanomas bearing shared or cross-reactive HLA-A. *Cell Immunol.*, 139: 411–425, 1992.
- Hayashi, Y., Hoon, D. S. B., Foshag, L. J., Park, M. S., Terasaki, P. I., and Morton, D. L. A preclinical model to assess the antigenicity of an HLA-A2 melanoma cell vaccine. *Cancer (Phila.)*, 72: 750–759, 1993.
- Pross, H. F., and Maroun, J. A. The biological response modifiers. *J. Immunol. Methods*, 68: 235–249, 1984.
- Hayashi, Y., Hoon, D. S. B., Park, M. S., Terasaki, P. I., and Morton, D. L. Cytotoxic T cell lines recognize autologous and allogeneic melanomas with shared or cross-reactive HLA-A. *Cancer Immunol. Immunother.*, 34: 419–423, 1992.
- Morton, D. L., Nizze, J. A., Hoon, D. S. B., Foshag, L. J., Famatiga, E., Gupta, R. K., Irie, R. F., and Rammung, K. P. Improved survival of advanced stage IV melanoma following active immunotherapy: correlation with immune response to melanoma vaccine. *Proc. Am. Soc. Clin. Oncol.*, 12: 391, 1993.
- McCue, C. S., O'Donnell, R. W., Marquis, D. M., and Sahasrabudhe, D. M. Renal cell carcinoma treated by vaccines for active specific immunotherapy: correlation of survival with skin testing by autologous tumor cells. *Cancer Immunol. Immunother.*, 32: 62–66, 1990.
- Bloemena, E., Gall, H., Ransom, J. H., Pamato, N., Murray, J. H., Bos, E., Schepers, R. J., Meijer, C. J., Hanna, M. G., and Vermorken, J. B. Delayed-type hypersensitivity reactions to tumor-associated antigens in colon carcinoma patients immunized with an autologous tumor cell/Bacillus Calmette-Guérin vaccine. *Cancer Res.*, 53: 456–459, 1993.
- O'Neil, B., Kawakami, Y., Restifo, N. P., Bennink, J. R., Yewdell, J. W., and Rosenberg, S. A. Detection of shared MHC-restricted human melanoma antigens after vaccinia virus-mediated transduction of genes coding for HLA. *J. Immunol.*, 151: 1410–1418, 1993.
- Darrow, T. L., Slingluff, C. L., and Seigler, H. F. The role of HLA class I antigens in recognition of melanoma cells by tumor-specific cytotoxic T lymphocytes: evidence for shared tumor antigens. *J. Immunol.*, 142: 3329–3335, 1989.
- Van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den Eynde, B., Knuth, A., and Boon, T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science (Washington DC)*, 254: 1643–1645, 1991.
- Lehmann, P. V., Sercarz, E. E., Forsthuber, T., Dayan, C. M., and Gammon, G. Determinant spreading and the dynamics of the autoimmune T-cell repertoire. *Immunol. Today*, 5: 203–208, 1993.
- Mukherji, B., Wilhelm, S. A., Guba, A., and Ergin, M. T. Regulation of cellular immune response against autologous human melanoma. Evidence for cell-mediated suppression of *in vitro* cytotoxic immune response. *J. Immunol.*, 136: 1888–1892, 1986.
- Hoon, D. S. B., Foshag, L. J., Nizze, J. A., Bohmann, R., and Morton, D. L. Suppressor cell activity in a randomized trial of patients receiving active specific immunotherapy with melanoma cell vaccine and low dosages of cyclophosphamide. *Cancer Res.*, 50: 5358–5364, 1990.
- Falk, K., Rotzschke, O., Stefanovic, S., Jung, G., and Rammensee, H. G. Allele-specific motifs revealed by sequencing self peptides eluted from MHC molecules. *Nature (Lond.)*, 351: 290–296, 1991.

**PubMed** PubMed QUERYOther Formats:  Links: ☐ Order this document*Cancer* 1992 Mar 1;69(5):1157-1164

## Relationship between immune response to melanoma vaccine immunization and clinical outcome in stage II malignant melanoma.

Bystryn JC, Oratz R, Roses D, Harris M, Henn M, Lew R

Department of Dermatology, New York University School of Medicine, NY 10016.

The authors investigated whether there was a relationship between the induction of a delayed-type hypersensitivity (DTH) response to melanoma vaccine immunization and disease recurrence. They studied prospectively 94 evaluable patients with surgically resected Stage II malignant melanoma who were immunized to a partially purified, polyvalent, melanoma antigen vaccine. The DTH response to skin tests to the vaccine was measured before treatment and at the fourth vaccine immunization. Vaccine treatment induced a strong DTH response in 29 (31%) patients, an intermediate response in 24 (25%), and no response in 41 (44%). The median disease-free survival (DFS) of patients with a strong, intermediate, and no DTH response to vaccine immunization was more than 72 months, 24 months, and 15 months, respectively. The relationship between an increase in the DTH response and a prolonged DFS was statistically significant ( $P = 0.02$ ); clinically meaningful (the median DFS of patients with a strong DTH response was 4.7 years longer than that of nonresponders); and, by multivariate analysis, independent of disease severity or overall immune competence. These findings suggest, but do not prove, that vaccine treatment can slow the progression of melanoma in some patients.

PMID: 1739915, UI: 92154561

 the above report in   format documents on this page through Loansome Doc

NCBI PubMed PubMed QUERY PubMed ?

Other Formats: Citation MEDLINE

Links: Related Articles

☐ Order this document*J Clin Oncol* 1997 Jun;15(6):2359-2370

## Autologous hapten-modified melanoma vaccine as postsurgical adjuvant treatment after resection of nodal metastases.

Berd D, Maguire HC Jr, Schuchter LM, Hamilton R, Hauck WW, Sato T, Mastrangelo MJ

Department of Medicine, Thomas Jefferson University, Philadelphia, PA 19107, USA.  
d\_berd@lac.jci.tju.edu

**PURPOSE:** To determine whether treatment with an autologous whole-cell vaccine modified with the hapten dinitrophenyl (DNP vaccine) is an effective postsurgical adjuvant treatment for melanoma patients with clinically evident nodal metastases. **PATIENTS AND METHODS:** Eligible patients had regional nodal metastases that were large enough ( $>$  or  $=$  3 cm diameter) to prepare vaccine. Following standard lymphadenectomy, patients were treated with DNP vaccine on a monthly or weekly schedule. **RESULTS:** Of 62 patients with metastasis in a single lymph node bed (stage III), 36 are alive after a median follow-up time of 55 months (range, 29 to 76); the projected 5-year relapse-free and overall survival rates are 45% and 58%, respectively. Of 15 patients with metastases in two nodal sites, five are alive with a median follow-up time of 73 months. An unexpected finding was the significantly better survival of older patients; the projected 5-year survival of patients greater than 50 versus  $\leq$  50 years was 71% and 47%, respectively ( $P = .011$ , log-rank test). The development of a positive delayed-type hypersensitivity (DTH) response to unmodified autologous melanoma cells was associated with significantly longer 5-year survival (71% v 49%;  $P = .031$ ). Finally, the median survival time from date of first recurrence was significantly longer for patients whose subcutaneous recurrence exhibited an inflammatory response ( $> 19.4$  v 5.9 months;  $P < .001$ ). **CONCLUSION:** Postsurgical adjuvant therapy with autologous DNP-modified vaccine appears to produce survival rates that are markedly higher than have been reported with surgery alone. Moreover, this approach has some intriguing immunobiologic features that might provide insights into the human tumor-host relationship.

### Publication Types:

- Clinical trial

PMID: 9196151, UI: 97339552

Save the above report in Macintosh Text format

Order documents on this page through Loansome Doc

NCBI PubMed PubMed QUERY PubMed ?

Other Formats: Citation MEDLINE

Links: Related Articles

☐ Order this document*Cancer Immunol Immunother* 1990;32(1):62-66

## Renal cell carcinoma treated by vaccines for active specific immunotherapy: correlation of survival with skin testing by autologous tumor cells.

McCune CS, O'Donnell RW, Marquis DM, Sahasrabudhe DM

University of Rochester Cancer Center, University of Rochester School of Medicine and Dentistry, New York 14642.

Eighteen patients with metastatic renal cell carcinoma, who were treated by vaccines for active specific immunotherapy, also completed skin testing with autologous tumor cells, both prior to and following vaccine treatment. All patients have now been followed for more than 5 years. Ten patients who remained skin-test-negative following treatment had no clinical responses, and all had expired by 22 months. Eight patients became skin-test-positive; three of these had clinical regressions and three remain alive after more than 69 months. The survival times of the skin-test-positive group were significantly superior to those of the skin-test-negative group. The results suggest that skin testing with autologous tumor cells may accurately identify those patients who have acquired antigen-specific cell-mediated antitumor immunity.

### Publication Types:

- Clinical trial

PMID: 2289200, UI: 91145924

 the above report in   format documents on this page through Loansome Doc

## ORIGINAL ARTICLE

Chungwen Wei · Eugene Storozynsky  
Alexander J. McAdam · Kun-Yun Yeh  
Brian R. Tilton · Richard A. Willis · Richard K. Barth  
R. John Looney · Edith M. Lord · John G. Frelinger

## Expression of human prostate-specific antigen (PSA) in a mouse tumor cell line reduces tumorigenicity and elicits PSA-specific cytotoxic T lymphocytes

Received: 4 April 1996 / Accepted: 31 May 1996

**Abstract** Human prostate-specific antigen (PSA) has a highly restricted tissue distribution. Its expression is essentially limited to the epithelial cells of the prostate gland. Moreover, it continues to be synthesized by prostate carcinoma cells. This makes PSA an attractive candidate for use as a target antigen in the immunotherapy of prostate cancer. As a first step in characterizing the specific immune response to PSA and its potential use as a tumor-rejection antigen, we have incorporated PSA into a well-established mouse tumor model. Line 1, a mouse lung carcinoma, and P815, a mouse mastocytoma, have been transfected with the cDNA for human PSA. Immunization with a PSA-expressing tumor cell line demonstrated a memory response to PSA which protected against subsequent challenge with PSA-expressing, but not wild-type, tumors. Tumor-infiltrating lymphocytes could be isolated from PSA-expressing tumors grown in naive hosts and were specifically cytotoxic against a syngeneic cell line that expressed PSA. Immunization with tumor cells resulted in the generation of primary and memory cytotoxic T lymphocytes (CTL) specific for PSA. The isolation of PSA-specific CTL clones from immunized animals further demonstrated that PSA can serve as a target antigen for antitumor CTL. The immunogenicity studies carried out in this mouse tumor model provide a rationale for the design of methods to elicit PSA-specific cell-mediated immunity in humans.

**Key words** Prostate-specific antigen · Prostate adenocarcinoma · T-cell-mediated immunity · Immunotherapy

### Introduction

Prostate cancer is the most frequently diagnosed malignancy and is the second leading cause of cancer death among American men [4–6]. If the cancer is detected early and is localized within the prostatic capsule, it can be cured by surgery [8]. However, the prognosis is often poor if metastasis has already occurred at the time of diagnosis [6, 23]. The mainstay of therapy for metastatic prostate cancer is androgen ablation accomplished by either androgen antagonistic agents or castration [15]. Although androgen withdrawal prolongs the period free of disease progression prostate tumor cells eventually become independent of androgen, resulting in a relapse [10, 22]. Chemotherapy specifically targeted to the androgen-independent prostate tumor cells, when combined with androgen ablation, has shown little success in a series of animal studies [13, 14, 16], and there are currently no effective chemotherapeutic agents that can control the growth of androgen-independent prostate tumor cells in human [36].

Given the profound medical impact of prostate cancer and the lack of adequate therapies, there is a need to develop new modalities of treatment. Immunotherapy strategies designed to engender a cellular immune response have recently received much attention and represent a promising approach for the treatment of many cancers [24, 32]. The benefit of this approach is that potent, long lasting immunity may be generated in the form of cytotoxic T cells (CTL) that will specifically lyse tumor cells. Such a response could be effective even against metastatic cells since T cells can circulate widely in humans. Nevertheless antigen-specific immunotherapy has been hampered by the difficulty in identifying antigens capable of inducing cell-mediated immunity. Tumor-specific antigens would presumably have to be derived from proteins expressed only in tumor cells or from mutated variants of normal proteins

C. Wei · R.K. Barth · E.M. Lord · J.G. Frelinger (✉)  
Cancer Center Immunology Unit, Box 704, University of Rochester,  
Rochester, NY 14642, USA  
Fax: (716) 461-4019

E. Storozynsky · A.J. McAdam · K.-Y. Yeh · B.R. Tilton ·  
R.A. Willis · R.K. Barth · E.M. Lord · J.G. Frelinger  
Department of Microbiology and Immunology,  
University of Rochester, Rochester, NY 14642, USA

R.J. Looney  
Department of Medicine, University of Rochester, Rochester,  
NY 14642, USA

*Present address:*

<sup>1</sup> Department of Pathology,  
Brigham and Women's Hospital, Boston, MA 02115, USA

These proteins are extremely rare and require laborious methods for their isolation, and in the case of mutant proteins may be different among individual cases. However, previous studies have shown that tyrosinase, a self-antigen in melanocytes, can be a target for anti-melanoma CTL [7], indicating that expression of a protein exclusively in tumor cells may not be a prerequisite for use as a tumor antigen. This may be especially true in cases such as prostate cancer in that the normal tissue from which the tumor is derived is non-essential and, therefore, may be safely ablated. This raises the possibility that prostate-specific antigen (PSA) might serve as a potential target antigen for prostate tumor immunotherapy.

PSA, a chymotrypsin-like serine protease, has a highly restricted tissue distribution and is expressed in the epithelial cells of the prostate gland, the same cell type from which most prostate tumors arise (reviewed in [34]). Indeed, PSA is widely used as a serum marker for prostate cancer [29]. Its expression is regulated by androgen, and it is present at extremely low levels in the circulation of adult men [30]. Most prostate tumors, even the poorly differentiated ones, continue to express PSA [41]. This cell-type-specific expression of PSA makes it a potential target antigen for antitumor CTL. As an initial step to examine the feasibility of eliciting a CTL response against PSA in humans, we established PSA-expressing mouse tumor cell lines, evaluated the immunogenicity of PSA expressed in the tumor cells in syngeneic mice, and analyzed the primary, memory and clonal CTL responses induced by PSA.

## Materials and methods

### Construction of PSA expression vector

PSA cDNA was isolated via reverse transcriptase/polymerase chain reaction [19] as follows: mRNA was isolated from the human prostate cell line LNCaP and reverse-transcribed into cDNA, which then served as the template in a polymerase chain reaction using PSA-specific oligonucleotides as primers. The sequences of the primers were

5' primer: 5'-ATCGAGTCGACGTCACCATGTGGGTCCCG3'.

3' primer: 5'-ACCTGAAGCTTTCAGGGGTTGGCCACGAT3'.

The PCR product was then subcloned into the pBluescript vector (Stratagene, LaJolla, Calif.) through the *SaI*I and *Hind*III sites designed in the primers. Clones were isolated and analyzed by restriction mapping and DNA sequencing. PSA cDNA was then excised from the pBluescript vector and subcloned into pH $\beta$ -Apri-neo, a eukaryotic expression vector, at the *SaI*I and *Hind*III sites (pH $\beta$ -PSA).

### Cell lines and PSA-expressing transfectants

Line 1, a BALB/cByJ (H-2<sup>d</sup>) mouse lung carcinoma cell line [44], and P815, a DBA/2 (H-2<sup>d</sup>) mastocytoma cell line [25], were grown in Excell 300 medium (JRH Biologics, Lenexa, Kan.) supplemented with 100 units/ml penicillin, 100  $\mu$ g/ml streptomycin and 2% fetal bovine serum. Line 1 and line 1/PSA cells were induced to express class I MHC by treatment with 3% dimethylsulfoxide (DMSO; Sigma, St. Louis, Mo.) for 5–20 days. To establish PSA-expressing cell lines,  $2 \times 10^5$  cells were transfected with 1  $\mu$ g pH $\beta$ -PSA plasmid DNA using lipofectin (BRL, Gaithersburg, Md.), and G418 (Gibco, Grand Island, N.Y.) resistant cells were cloned by limiting dilution. Clones were

expanded and screened for PSA production by a sandwich enzyme-linked immunosorbent assay (ELISA).

### PSA ELISA

To analyze the expression of PSA by the transfectants,  $1 \times 10^6$  cells were plated and allowed to grow in 2 ml medium for 48 h before culture supernatants were harvested. PSA concentrations in the supernatants were then quantified by a sandwich ELISA using two mouse monoclonal antibodies, RLSD06 and RLSD09 (ATCC HB8527 and HB8525, Rockville, Md.), specific for human PSA. Then 96-well plates were coated with 0.1 ml RLSD06 (0.1 mg/ml in PBS) at 4 °C overnight, and washed three times with phosphate-buffered saline (PBS)/0.2% Tween 20. Plates were then blocked with Biotin-Tween at 37 °C for 1 h, and washed three times; 0.1-ml samples of twofold serially diluted culture supernatants and PSA standard (25 ng/ml, Hybritech, San Diego, Calif.) were then added to the plates and incubated at 37 °C for 1 h. After washing with PBS, 0.1 ml biotin-conjugated RLSD09 (10  $\mu$ g/ml) was added and incubated for another 1 h at 37 °C. Following washes with PBS, streptavidin-conjugated horseradish peroxidase (1:500 in PBS, Jackson ImmunoResearch, West Grove, Pa.) was added. Plates were then washed and *o*-phenylenediamine (Sigma, St. Louis, Mo.) was added as the chromogen. The absorbance at 492 nm was determined using an ELISA reader.

### In vivo tumor growth

Groups of 5 BALB/cByJ (BALB/c) mice (Jackson Laboratory, Bar Harbor, Me.) at 2–4 months of age were either used without immunization (unprimed) or primed twice at 10-day intervals with  $5 \times 10^6$  irradiated P815/PSA or parental P815 cells i.p. and then, 10 days after boosting, challenged i.m. with  $1 \times 10^4$  viable line 1 or line 1/PSA cells. Tumor-bearing legs were measured twice a week with vernier calipers, and tumor size was calculated as the square root of two perpendicular diameters. Tumors were allowed to grow until the mean thigh diameter reached 15 mm, at which point the animals were sacrificed. Animals rejecting tumors were measured for at least 100 days following tumor challenge. A  $\chi^2$  analysis was then used to determine the significance of difference in tumor incidence among experimental groups.

### Isolation of tumor-infiltrating lymphocytes

Samples containing  $2 \times 10^5$  line 1/PSA or parental line 1 cells in 50  $\mu$ l were injected i.m. into the thighs of syngeneic BALB/c mice. Tumors were allowed to grow for 20 days before they were removed and dissociated with 10 ml 0.2% collagenase (Sigma, St. Louis, Mo.)/g tumor tissue at 37 °C for 75 min [35]. Thy1-expressing cells were enriched using sheep anti-(rat-IgG) paramagnetic beads (Dyna, Great Neck, N.Y.) coupled to T24/40.7, a rat anti-(mouse-Thy1) monoclonal antibody, as described [27, 43]. Tumor-infiltrating lymphocytes were cultured for 12–16 h in Excell medium supplemented with 5% fetal bovine serum and 10% EL4 supernatant, and then used as effector cells in the cytotoxicity assay against target cells. Culture with EL4 supernatant has been shown to have no effect on cytotoxicity (unpublished observations).

### Generation of PSA-specific CTL clones

Class-I-high (DMSO-induced) and irradiated line 1/PSA or line 1 cells ( $1 \times 10^7$  cells/sample) were injected i.p. into BALB/c mice, which were reinjected after 2 weeks. Two weeks later, mice were sacrificed and their spleens removed. The dissociated splenocytes were restimulated for 5 days in vitro and the cytotoxicity was assayed against line 1 and P815 cells that were either untransfected or transfected with pH $\beta$ -PSA. PSA-specific CTL clones were isolated from the bulk splenocyte cultures by limiting dilution as described [3].

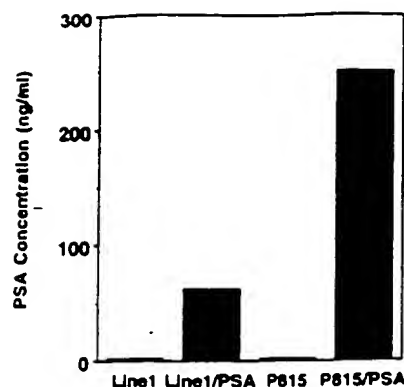


Fig. 1 Expression of prostate-specific antigen (PSA) in transfected line 1 and P815 cells. Samples comprising  $1 \times 10^6$  cells were plated in 2 ml medium and allowed to grow for 48 h before the supernatants were collected. Twofold serially diluted culture supernatants (100  $\mu$ l) from the parental or PSA-transfected line 1 and P815 cells and PSA standard (25 ng/ml at 1:1 dilution) were assayed by a sandwich enzyme-linked immunosorbent assay using mAb RLSD06 and RLSD09 as described in Materials and methods. By comparison to the PSA standard, the absorbances (at 492 nm) were then converted to concentrations (ng/ml)

#### <sup>51</sup>Cr-release cytotoxicity assay

Six-hour cytotoxicity assays against <sup>51</sup>Cr-labeled target cells (2000/well) were performed at various effector-to-target ratios as previously described [26]. The percentage specific lysis was calculated as  $[(\text{experimental release} - \text{spontaneous release}) / (\text{maximal release} - \text{spontaneous release})] \times 100$ . Spontaneous release was never more than 20% of the maximal release for any target.

## Results

### Generation of PSA-expressing line 1 and P815 tumor cell lines

In order to determine whether PSA can elicit specific T-cell-mediated immunity in mice, two different mouse tumor cell lines of the same haplotype that express PSA were established. The use of two unrelated, PSA-expressing cell lines allows us to elicit responses with one line and to test the effectors on the other in order to ensure that any cytotoxic response is PSA-specific. Line 1 lung carcinoma and P815 mastocytoma cells (H-2<sup>d</sup>) were transfected with the pH $\beta$ -Apr1-neo expression vector harboring the human PSA cDNA, and G418 resistant cells were cloned by limiting dilution. The expression of PSA in the isolated clones was analyzed by determining the PSA concentrations in the culture supernatants by ELISA. By comparison to the PSA standard, it has been shown that both the isolated PSA-transfected line 1 and P815 clones, but not the parental lines, were actively synthesizing and secreting PSA equivalent to about 150 ng/ $10^6$  cells and 500 ng/ $10^6$  cells respectively in a 48-h period (Fig. 1). The levels of PSA expression in these cell lines are comparable to what has been observed in the dihydrotestosterone-induced human prostate cell line LNCaP [11, 21], and are somewhat

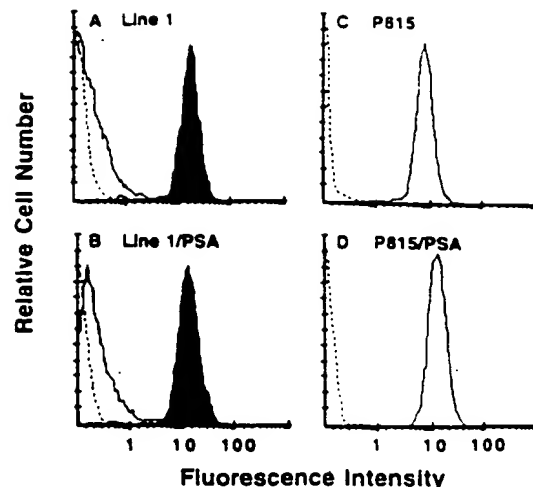


Fig. 2A-D Effect of transfection and expression of PSA on class I expression. (A) Line 1 and (B) line 1/PSA cells, either untreated or treated with 3% dimethylsulfoxide (DMSO) for 7 days, along with (C) P815 and (D) P815/PSA cells were stained for class I using AF4-62.4 (anti-H-2D<sup>d</sup>), followed by fluorescein-isothiocyanate-conjugated goat anti-mouse IgG as described previously [35]. - - Staining with the second-step antibody only, white areas staining for class I (uninduced), shaded areas class I (DMSO-induced)

higher than that seen in the previously reported PSA-transfected mouse colon adenocarcinoma cell line MC-38 [17].

Since we wished to examine the specific cytotoxic T cell response to PSA, it was essential to measure the levels of class I MHC to ensure that expression of the transgene did not alter the class I expression in either of these PSA-transfected tumor cell lines. As shown previously, line 1 cells normally express very low levels of class I molecules. However, the expression of class I in line 1 cells can be up-regulated 30- to 50-fold by growth *in vivo* or by treatment with either DMSO or interferon  $\gamma$  *in vitro* [1, 28]. As expected, PSA-transfected line 1 cells (line 1/PSA) also expressed low levels of class I, and treatment with DMSO induced class I expression to a level comparable to that of DMSO-induced line 1 cells (Fig. 2A, B). It was also shown that parental P815 and PSA-transfected P815 cells (P815/PSA) had similar constitutive levels of class I expression (Fig. 2C, D). Hence, transfection with PSA did not alter the class I expression in either cell line, nor the inducibility of class I expression in line 1/PSA cells.

### Priming with PSA-expressing tumor cells induces specific tumor rejection

To assess the immunogenicity of PSA expressed in the mouse tumor cells, we examined the ability of syngeneic BALB/c mice to reject PSA-expressing tumors with or without relevant priming. By immunizing mice with one tumor cell line and challenging with a viable, unrelated tumor cell line of the same haplotype, the effects of PSA expression on tumor rejection can be determined in the absence of background caused by an antitumor response.

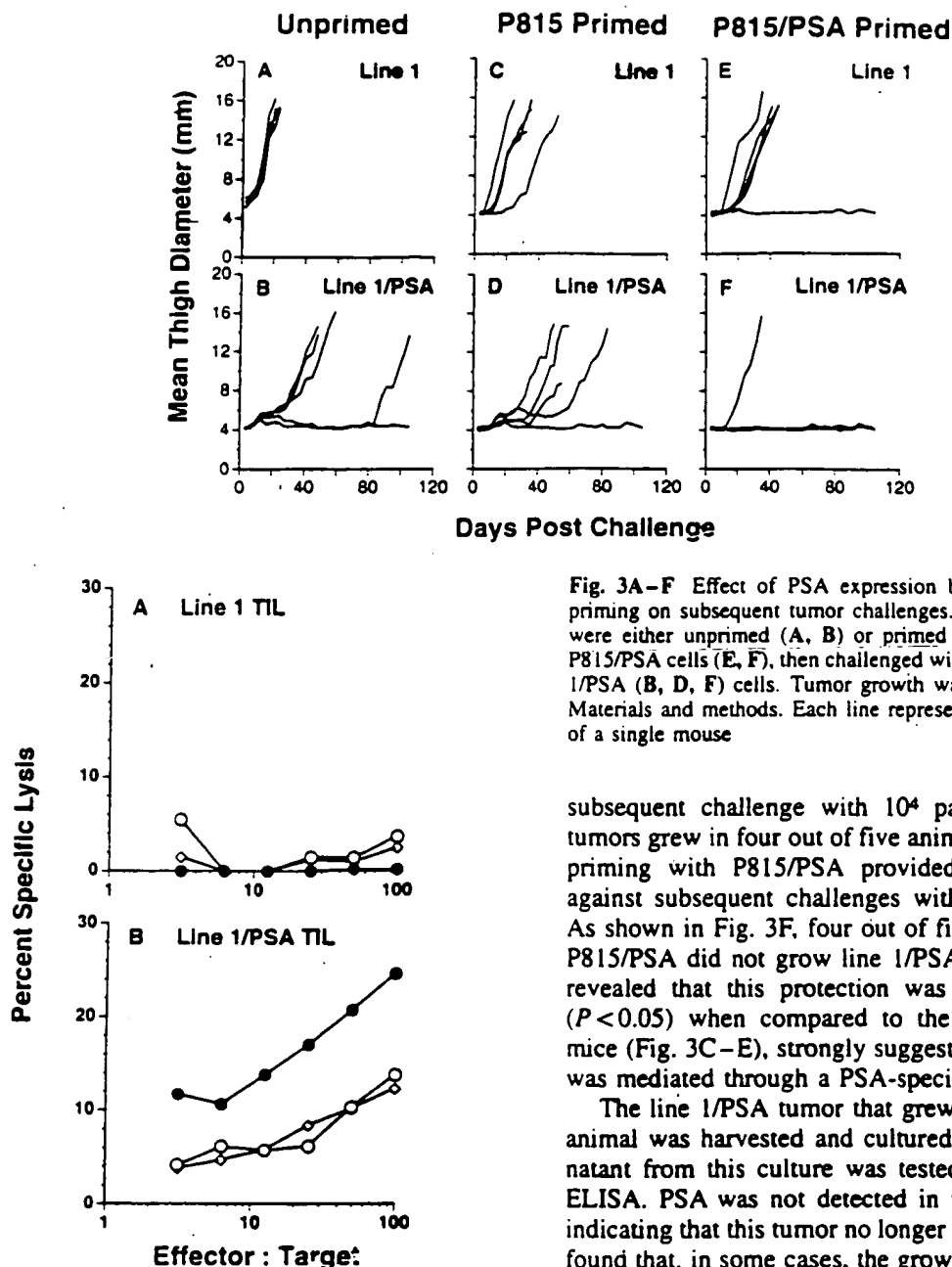


Fig. 3A-F Effect of PSA expression by the tumor cells used for priming on subsequent tumor challenges. Groups of 5 BALB/c mice were either unprimed (A, B) or primed twice with P815 (C, D) or P815/PSA cells (E, F), then challenged with  $10^4$  line 1 (A, C, E) or line 1/PSA (B, D, F) cells. Tumor growth was measured as described in Materials and methods. Each line represents the mean thigh diameter of a single mouse

subsequent challenge with  $10^4$  parental line 1 cells, as tumors grew in four out of five animals (Fig. 3E). However, priming with P815/PSA provided significant protection against subsequent challenges with  $10^4$  line 1/PSA cells. As shown in Fig. 3F, four out of five animals primed with P815/PSA did not grow line 1/PSA tumors. A  $\chi^2$  analysis revealed that this protection was statistically significant ( $P < 0.05$ ) when compared to the other three groups of mice (Fig. 3C-E), strongly suggesting that tumor rejection was mediated through a PSA-specific immune response.

The line 1/PSA tumor that grew in a P815/PSA-primed animal was harvested and cultured in vitro and the supernatant from this culture was tested in the PSA sandwich ELISA. PSA was not detected in the culture supernatant, indicating that this tumor no longer expressed PSA. We also found that, in some cases, the growth of line 1/PSA tumors in P815-primed animals resulted in a cell line that no longer expressed PSA (data not shown). Whether this is due to selection of a negative variant in vivo or to another form of antigenic variation related to the instability of the transfected gene is not clear.

#### PSA-expressing tumors induce a PSA-specific CTL response in unimmunized mice

Line 1/PSA tumors appeared to establish later than the parental line 1 tumors in both naive animals (Fig. 3A, B) and mice primed with P815 (Fig. 3C, D). This was not due to an intrinsic difference in the growth rates of the cells, as both line 1 and line 1/PSA exhibited the same growth rates in vitro with a doubling time of approximately 13 h (data

Fig. 4A,B Characterization of tumor-infiltrating lymphocytes isolated from line 1 and line 1/PSA tumors. Tumor-infiltrating lymphocytes isolated from mice inoculated with (A) line 1 (line 1 tumor-infiltrating lymphocytes, TIL) or (B) line 1/PSA (line 1/PSA TIL) were tested in a 6-h cytotoxicity assay against DMSO-induced line 1 (○), line 1/PSA (○) and DMSO-induced line 1/PSA (●) cells

Naive animals and animals immunized with irradiated P815 or P815/PSA cells were challenged 10 days later with viable line 1 or line 1/PSA cells. In unprimed and P815-primed control animals, tumors formed in all the animals challenged with  $10^4$  parental line 1 cells, and in four out of five animals challenged with the same dose of line 1/PSA cells (one mouse with tumor died prematurely at day 55 in the P815-primed group, Fig. 3A-D). As expected, immunization with P815/PSA offered no protection against a



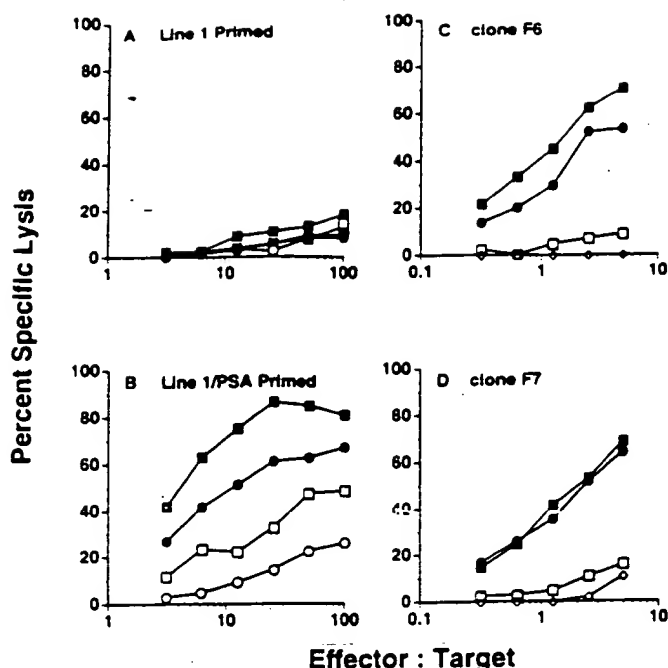


Fig. 5 Characterization of splenocytes and isolation of cytotoxic T lymphocyte (CTL) clones specific for PSA. Splenocytes isolated from mice immunized with (A) line 1 or (B) line 1/PSA, and (C, D) two CTL clones isolated from the bulk splenocyte cultures were tested in a 6-h cytotoxicity assay against DMSO-induced line 1 ( $\diamond$ ), line 1/PSA ( $\circ$ ), DMSO-induced line 1/PSA ( $\bullet$ ), P815 ( $\square$ ) and P815/PSA ( $\blacksquare$ ) cells

not shown). Since transfection of line 1 cells with PSA did not change the inducibility of class I expression *in vitro* (Fig. 2A, B) or *in vivo* (data not shown), the retarded growth of line 1/PSA cells in naive and P815-primed animals suggested the induction of a primary anti-PSA immune response. To analyze directly whether such a primary response against PSA was occurring, naive syngeneic BALB/c mice were inoculated with line 1/PSA or parental line 1 cells and tumors were allowed to grow. Tumor-infiltrating lymphocytes (TIL) were harvested 20 days after implantation, and the cytolytic activity of the TIL was determined by 6-h  $^{51}\text{Cr}$ -release assays against DMSO-induced line 1/PSA (class-I-high), line 1/PSA (class-I-low) and parental DMSO-induced line 1 (class-I-high) cells. The inducibility of class I expression in line 1/PSA cells provides an internal control for whether the PSA-induced immune response is due to class-I-restricted CTL. As shown in Fig. 4, TIL isolated from line 1/PSA tumors, but not from parental line 1 tumors, contained PSA-specific CTL. The cytolytic activity of the PSA-specific CTL depended on the expression of class I molecules as well as PSA, as neither uninduced line 1/PSA nor DMSO-induced line 1 cells could serve as targets (Fig. 4). These results indicate that PSA elicits a specific, class-I-restricted primary CTL response in mice. The generation of a primary CTL response against PSA may, in part, cause the delay in growth of line 1/PSA tumors in naive and P815-primed animals as observed in Fig. 3.

Immunization with a PSA-expressing tumor results in PSA-specific memory CTL

The observation that expression of PSA in the primary tumor elicited rejection of subsequent challenges with PSA-expressing tumors as well as the demonstration of TIL growing, PSA-expressing tumors prompted us to examine the memory response against PSA. BALB/c mice were primed twice with irradiated line 1 or line 1/PSA, and splenocytes were removed and re-stimulated again *in vitro* before their cytolytic activity was tested against target cells. Splenocytes isolated from mice immunized with line 1 cells showed no cytolytic activity against PSA-expressing cells (Fig. 5A). However, splenocytes from line 1/PSA-immunized mice lysed target cells in a PSA-dependent manner. As shown in Fig. 5B, these splenocytes were capable of lysing DMSO-induced line 1/PSA targets but not line 1/PSA (class-I-low) targets or DMSO-induced line 1 target (data not shown), demonstrating that the effectors were specific for PSA in a class-I-restricted fashion. Specificity for PSA was further shown by demonstrating that an unrelated syngeneic target cell, P815/PSA, could be lysed efficiently by these effectors while the parental line, P815, was lysed to a much lesser extent.

Although most of the lysis appeared to be PSA-specific, there were also significant levels of PSA-independent cytolytic activity in the bulk splenocyte cultures. To examine the specificity of the memory CTL response against PSA, the bulk splenocyte cultures were cloned by limiting dilution and PSA-specific CTL clones were isolated on the basis of their high lysis of PSA-expressing target cells. The specific cytolytic activity for PSA-expressing targets of two of these clones is shown in Fig. 5C, D. These clones showed highly efficient lysis of DMSO-induced line 1/PSA and P815/PSA targets, which express both PSA and class I, while there was no specific lysis of the class-I-expressing targets DMSO-induced line 1 and P815. The data from these cytotoxicity assays demonstrate the presence of a classical, class-I-restricted memory CTL response to PSA in immunized animals.

## Discussion

Immunotherapy for prostate tumors designed to promote cell-mediated immunity has recently been pursued. It has been shown that interleukin-2-transfected Dunning prostate carcinoma cells induce a local antitumor response in the rat model, which appears to be due to a non-T-cell mediated mechanism [31]. In the same Dunning rat model, granulocyte/macrophage-colony-stimulating-factor-transduced tumor vaccine cells have been shown to prolong survival in rats with established prostate tumor deposited at a distant site [38]. However, these reports neither characterized the antigens recognized nor demonstrated directly T-cell-mediated immune response. A recent report, which a PSA-transfected mouse colon adenocarcinoma cell line was shown to grow faster in athymic mice than

in syngeneic C57BL/6 mice, suggested the possible involvement of T cells in slowing the growth of PSA-expressing tumors [17]. However, the T cell response was not further characterized. Finally, a PSA-expressing vaccinia vector has recently been constructed in an effort to generate a PSA vaccine that could potentially be used in humans [12]. Inoculation of rhesus monkeys with this vaccinia vector resulted in a PSA-specific IgM response as well as T cell proliferation in response to PSA. Again, however, there was no further characterization of T cell subsets involved or whether the vaccine could engender an antitumor response.

In this report, we extended these studies by showing that the expression of human PSA in mouse tumor cells elicits a potent CTL response in mice. Immunization with a PSA-expressing tumor resulted in rejection of subsequent challenges with tumors that expressed PSA. The generation of PSA-specific CTL clones clearly is an indication of the ability of PSA to act as a target for antitumor CTL. Interestingly, the expression of a single foreign protein is sufficient to engender a potent T cell response and protect against an aggressive and poorly immunogenic tumor. The immunogenicity studies presented here thus serve as a basis for the design of methods to elicit a PSA-specific CTL response in humans. If a PSA-specific CTL response can be elicited in patients with prostate cancer, then an immune response could be directed to the tumor.

One obvious obstacle in eliciting an immune response to PSA is that humans are generally tolerant to self-antigens. However, the large number of tissue-specific autoimmune diseases in humans demonstrate that self-tolerance is not always absolute. Moreover, recent studies on human melanoma have shown that tyrosinase, Melan-A-MART-1, gp100 and gp75, all normal self-proteins specific to the melanocyte lineage, can serve as targets for anti-melanoma CTL [2, 7, 18, 42]. The existence of anti-melanoma CTL specific for self-proteins clearly demonstrates that self-antigens can be targets for CTL.

PSA offers an excellent opportunity to examine the ability of self-antigens to serve as targets for CTL. Antigens that are present at low levels or sequestered from the immune system [39] enhance the likelihood of unresponsiveness. Since PSA is highly restricted in its tissue expression and is expressed late in development and at low levels in circulation, it may be below the detection limit of the immune system. Furthermore, it has recently become apparent that sequestration need not be absolute. It may be sufficient that the target antigens are not presented in an immunogenic fashion. This phenomenon, termed clonal ignorance, has been experimentally demonstrated in elegant transgenic experiments using a lymphocytic choriomeningitis virus (LCMV) protein expressed in pancreatic islet cells [33]. These cells were tolerated until the LCMV protein was introduced in an immunogenic form by infecting the mice with LCMV, inducing T cell infiltration into pancreatic islets with resultant hypoglycemia. These findings suggest that the transgenic mice were not tolerant to the LCMV protein but merely ignorant of it. It is possible that non-responsiveness to PSA in humans might be similar. Indeed, the detection of anti-PSA antibodies in patients

with advanced prostate cancer [9], in which PSA expression is greatly increased, indicates that PSA can be the target of a humoral autoimmune response and that tolerance to PSA is not absolute. Since CD4 cells are required for efficient antibody production, this finding also suggests the presence of a CD4 T cell response.

There are also theoretical reasons why tolerance may not be absolute. While tolerance may be induced for certain epitopes of self-antigens, it is possible that there are other determinants present in the same self-antigen that have not induced self-tolerance (reviewed in [20, 32, 40]). In essence the immune system is ignorant of these "cryptic" epitopes. However, under certain circumstances, these epitopes can be displayed in an immunogenic form and induce an autoimmune response. For instance, it has been shown that binding of HIV gp120 to CD4 causes extensive internalization of this complex, which leads to increased presentation of CD4 epitopes on the MHC molecules of that cell [37]. This altered presentation leads to an increase in the presentation of a cryptic CD4 epitope and subsequent autoimmune-T-cell-mediated destruction of these cells. Therefore, even if humans are truly tolerant of the dominant antigenic peptides of PSA, the unresponsiveness to PSA as a whole may be reversed by presenting the cryptic determinants of PSA in an immunogenic form.

We are currently developing a transgenic mouse model in which human PSA is specifically expressed in the prostate gland. This model more closely approximates the situation in humans in that PSA becomes a self-antigen. By immunizing these mice with PSA-expressing tumor cells or by other methods, we will be able to evaluate the effect of endogenous PSA expression on the immune response to PSA. In our current work, we have shown that a strong immune response can be effective against tumors expressing PSA. Future studies with our transgenic mouse model will allow us to develop methods for generating an autoimmune, cell-mediated response against PSA that will be translatable to the immunotherapy of human prostate cancer.

**Acknowledgements** This work was supported in part by grant IM-493 (to E.M.L.) and grant IM-569 (to R.K.B.) from the American Cancer Society, by grant POAG104643A (to R.J.L.) and grant CA70218 (to J.G.F.) from the National Institutes of Health, and by a grant from the Pepper Center (to J.G.F.). C.W. was supported by NIH Postdoctoral Training Grant T32CA09363. A.J.M. was supported in part by grant T32GM07356 from the NIH. E.S. and R.A.W. were supported in part by grant T32AI07285 from the NIH. This is publication number 127 from the Immunology and Immunotherapy Unit of the University of Rochester Cancer Center.

## References

1. Bahler DW, Lord EM (1985) Dimethyl sulfoxide induces expression of H-2 antigens on mouse lung carcinoma cells. *J Immunol* 134:2790
2. Bakker AB, Schreurs MW, Boer AJ de, Kawakami Y, Rosenberg SA, Adema GJ, Figdor CG (1994) Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. *J Exp Med* 179:1005

3. Blieden TM, McAdam AJ, Freiler JG, Lord EM (1991) Mechanism of cytolytic T lymphocyte killing of a low class I-expressing tumor. *J Immunol* 147:1433
4. Boring CC, Squires TS, Tong T (1991) Cancer statistics. *CA Cancer J Clin* 41:19
5. Boring CC, Squires TS, Tong T (1993) Cancer statistics. *CA Cancer J Clin* 43:7
6. Brawer MK (1995) How to use prostate-specific antigen in the early detection or screening for prostatic carcinoma. *CA Cancer J Clin* 45:148
7. Brichard V, Van Pel A, Wolfel T, Wolfel C, De Plaen E, Lethe B, Coulier P, Boon T (1993) The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med* 178:489
8. Catalona WJ (1995) Surgical management of prostate cancer. *Cancer* 75:1903
9. Chu TM, Kuriyama M, Johnson E, Papsidero LD, Killian CS, Murphy GP, Wang MC (1984) Circulating antibody to prostate antigen in patients with prostate cancer. *Transplant Proc* 16:481
10. Crawford ED, Eisenberger MA, McLeod DG, Spaulding JT, Benson R, Dorr FA, Blumenstein BA, Davis MA, Goodman PJ (1985) A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. *N Engl J Med* 321:419
11. Guo Y, Pili R, Passaniti A (1994) Regulation of prostate-specific antigen gene expression in LNCaP human prostatic carcinoma cells by growth, dihydrotestosterone, and extracellular matrix. *Prostate* 24:1
12. Hodge JW, Schlom J, Donohue SJ, Tomaszewski JE, Wheeler CW, Levine BS, Gritz L, Panicali D, Kantor JA (1995) A recombinant vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate. *Int J Cancer* 63:231
13. Isaacs JT (1982) Hormonally responsive versus unresponsive progression of prostatic cancer to antiandrogen therapy as studied with the Dunning R-3327-AT and -G rat adenocarcinomas. *Cancer Res* 42:5010
14. Isaacs JT (1989) Relationship between tumor size and curability of prostatic cancer by combined chemo-hormonal therapy in rats. *Cancer Res* 49:6290
15. Isaacs JT (1994) Role of androgens in prostatic cancer. *Vitam Horm* 49:433
16. Isaacs JT, Hukku B (1988) Nonrandom involvement of chromosome 4 in the progression of rat prostatic cancer. *Prostate* 13:165
17. Karr JF, Kantor JA, Hand PH, Eggensperger DL, Schlom J (1995) The presence of prostate-specific antigen-related genes in primates and the expression of recombinant human prostate-specific antigen in a transfected murine cell line. *Cancer Res* 55:2455
18. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Rivolini L, Topalian SL, Miki T, Rosenberg SA (1994) Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc Natl Acad Sci USA* 91:3515
19. Kawasaki ES, Clark SS, Coyne MY, Smith SD, Champlin R, Witte ON, McCormick FP (1988) Diagnosis of chronic myeloid and acute lymphocytic leukemias by detection of leukemia-specific mRNA sequences amplified in vitro. *Proc Natl Acad Sci USA* 85:5698
20. Lanzavecchia A (1995) How can cryptic epitopes trigger autoimmunity? *J Exp Med* 181:1945
21. Lee C, Sutkowski DM, Sensibar JA, Zelter D, Kim I, Amsel I, Shaw N, Prins GS, Kozlowski JM (1995) Regulation of proliferation and production of prostate-specific antigen in androgen-sensitive prostatic cancer cells, LNCaP, by dihydrotestosterone. *Endocrinology* 136:796
22. Lepor H, Ross A, Walsh PC (1982) The influence of hormonal therapy on survival of men with advanced prostatic cancer. *J Urol* 128:335
23. Lerner SP, Seale-Hawkins C, Carlton CE, Jr., Scardino PT (1991) The risk of dying of prostate cancer in patients with clinically localized disease. *J Urol* 146:1040
24. Lotze MT, Finn OJ (1990) Recent advances in cellular immunology: implications for immunity to cancer. *Immunol Today* 11:190
25. Lundak RL, Freiler DJ (1973) Cellular immune response against tumor cells. I. In vitro immunization of allogeneic and syngeneic mouse spleen cell suspensions against DBA mastocytoma cells. *Cell Immunol* 9:60
26. Maryanski JL, Van Snick J, Cerottini JC, Boon T (1982) Immunogenic variants obtained by mutagenesis of mouse mastocytoma P815. III. Clonal analysis of the syngeneic cytolytic T lymphocyte response. *Eur J Immunol* 12:401
27. McAdam AJ, Felcher A, Woods ML, Pulaski BA, Hutter EK, Frelinger JG, Lord EM (1994) Transfection of transforming growth factor-beta producing tumor EMT6 with interleukin-2 elicits tumor rejection and tumor reactive cytotoxic T-lymphocytes. *J Immunother* 15:155
28. McAdam AJ, Pulaski BA, Storozynsky E, Yeh KY, Sickel JZ, Frelinger JG, Lord EM (1995) Analysis of the effect of cytokines (interleukins 2, 3, 4, and 6, granulocyte-macrophage colony-stimulating factor, and interferon-gamma) on generation of primary cytotoxic T lymphocytes against a weakly immunogenic tumor. *Cell Immunol* 165:183
29. McCormack RT, Rittenhouse HG, Finlay JA, Sokoloff RL, Wang TJ, Wolfert RL, Lilja H, Oesterling JE (1995) Molecular forms of prostate-specific antigen and the human kallikrein gene family: a new era. *Urology* 45:729
30. Montgomery BT, Young CY, Bilhartz DL, Andrews PE, Prescott JL, Thompson NF, Tindall DJ (1992) Hormonal regulation of prostate-specific antigen (PSA) glycoprotein in the human prostatic adenocarcinoma cell line, LNCaP. *Prostate* 21:63
31. Moody DB, Robinson JC, Ewing CM, Lazenby AJ, Isaacs WB (1994) Interleukin-2 transfected prostate cancer cells generate a local antitumor effect in vivo. *Prostate* 24:244
32. Nanda NK, Sercarz EE (1995) Induction of anti-self-immunity to cure cancer. *Cell* 82:13
33. Ohashi PS, Oehen S, Buerki K, Pircher H, Ohashi CT, Odermatt B, Malissen B, Zinkernagel RM, Hengartner H (1991) Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* 65:305
34. Peehl DM (1995) Prostate specific antigen role and function. *Cancer* 75 [Suppl]:2021
35. Pulaski BA, McAdam AJ, Hutter EK, Biggar S, Lord EM, Frelinger JG (1993) Interleukin 3 enhances development of tumor-reactive cytotoxic cells by a CD4-dependent mechanism. *Cancer Res* 53:2112
36. Raghavan D (1988) Non-hormone chemotherapy for prostate cancer: principles of treatment and application to the testing of new drugs. *Semin Oncol* 15:371
37. Salemi S, Caporossi AP, Boffa L, Longobardi MG, Barnaba V (1995) HIVgp120 activates autoreactive CD4-specific T cell responses by unveiling of hidden CD4 peptides during processing. *J Exp Med* 181:2253
38. Sanda MG, Ayyagari SR, Jaffee EM, Epstein JI, Clift SL, Cohen LK, Dranoff G, Pardoll DM, Mulligan RC, Simons JW (1994) Demonstration of a rational strategy for human prostate cancer gene therapy. *J Urol* 151:622
39. Schwartz RH (1993) Immunological tolerance. In: Paul WE (ed) *Fundamental immunology*, 3rd edn. Raven, New York, p 677
40. Sercarz EE, Lehmann PV, Ametani A, Benichou G, Miller A, Moudgil K (1993) Dominance and crypticity of T cell antigenic determinants. *Annu Rev Immunol* 11:729
41. Sinha AA, Hagen KA, Sibley RK, Wilson MJ, Limas C, Reddy PK, Blackard CE, Gleason DF (1986) Analysis of fixation effects on immunohistochemical localization of prostatic specific antigen in human prostate. *J Urol* 136:722
42. Wang RF, Robbins PF, Kawakami Y, Kang XQ, Rosenberg SA (1995) Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes. *J Exp Med* 181:799
43. Yang JC, Perry-Lalley D, Rosenberg SA (1990) An improved method for growing murine tumor-infiltrating lymphocytes with in vivo antitumor activity. *J Biol Response Mod* 9:149
44. Yuhas JM, Pazmino NH, Proctor JO, Toya RE (1974) A direct relationship between immune competence and the subcutaneous growth rate of a malignant murine lung tumor. *Cancer Res* 34:722

## Phase I Clinical Trial: T-Cell Therapy for Prostate Cancer Using Autologous Dendritic Cells Pulsed With HLA-A0201-Specific Peptides from Prostate-Specific Membrane Antigen

G. Murphy, B. Tjoa, H. Ragde, G. Kenny, and A. Boynton

Pacific Northwest Cancer Foundation, Cancer Research Division (G.M., B.T., H.R., G.K.),  
Department of Molecular Medicine (A.B.), Northwest Hospital, Seattle, Washington

**BACKGROUND.** Conventional treatment for metastatic prostate cancer have failed to demonstrate curative potential in all patients. Investigations involving the role of T-cell immunity in the clearance of neoplastic cells are now available. Development of T-cell immunotherapy may give a new approach to the treatment of advanced metastatic prostate cancer.

**METHODS.** A phase I clinical trial assessing the administration of autologous dendritic cells (DC) pulsed with HLA-A0201-specific prostate-specific membrane antigen (PSMA) peptides were conducted. Participants were divided into five groups receiving four or five infusions of peptides alone (PSM-P1 or PSM-P2; groups 1 and 2, respectively), autologous DC (group 3), or DC pulsed with PSM-P1 or P2 (groups 4 and 5, respectively).

**RESULTS.** No significant toxicity was observed in all five groups. Cellular response against PSM-P1 and -P2 was observed in HLA-A2<sup>+</sup> patients infused with DC pulsed with PSM-P1 or -P2 (groups 4 and 5), respectively. An average decrease in PSA was detected only in group 5. Seven partial responders were identified based on NPCP criteria + PSA.

**CONCLUSIONS.** Infusions of test substances were well tolerated by all study participants. Detection of cellular response and decrease in PSA level in some patients who received DC pulsed with PSM-P2 indicate this method's potential in prostate cancer therapy.

© 1996 Wiley-Liss, Inc.

**KEY WORDS:** dendritic cells (DC), T cell, immunotherapy, prostate cancer, Phase I trial

### INTRODUCTION

Immunotherapy has recently emerged as a promising new approach in cancer therapy, in light of new advances in two important components. Discovery of cancer-specific antigens (Ag), e.g., melanoma, breast, and ovarian cancer-specific/associated protein and peptides have provided the first component: targets for specific therapy [1-5]. T cells recognize processed peptide antigens bound to the major histocompatibility complex (MHC) proteins [6]. Previous studies have identified specific motifs of MHC class I-binding peptides [7-10]. These advances are useful for predicting potential T-cell epitopes from proteins of known amino acid sequences. HLA-A0201 (a subset of HLA-A2 family) is one of the MHC class I protein

expressed by a large proportion of the population [11]. Using the specific motif, we identified HLA-A0201-specific prostate-specific membrane antigen (PSMA) peptides for use as Ag in our prostate cancer immunotherapy study [12]. PSMA is a specific prostate antigen recognized by monoclonal antibody 7E11.C5 [13,14]. Levels of PSMA are elevated in the serum of hormone refractory advanced prostate cancer patients. PSMA is also found in normal male serum and seminal fluid, and in prostate epithelial cells [15,16].

Address reprint requests to Dr. Gerald P. Murphy, Pacific Northwest Cancer Foundation, Northwest Hospital, 120 Northgate Plaza, Suite 205, Seattle, WA 98125.

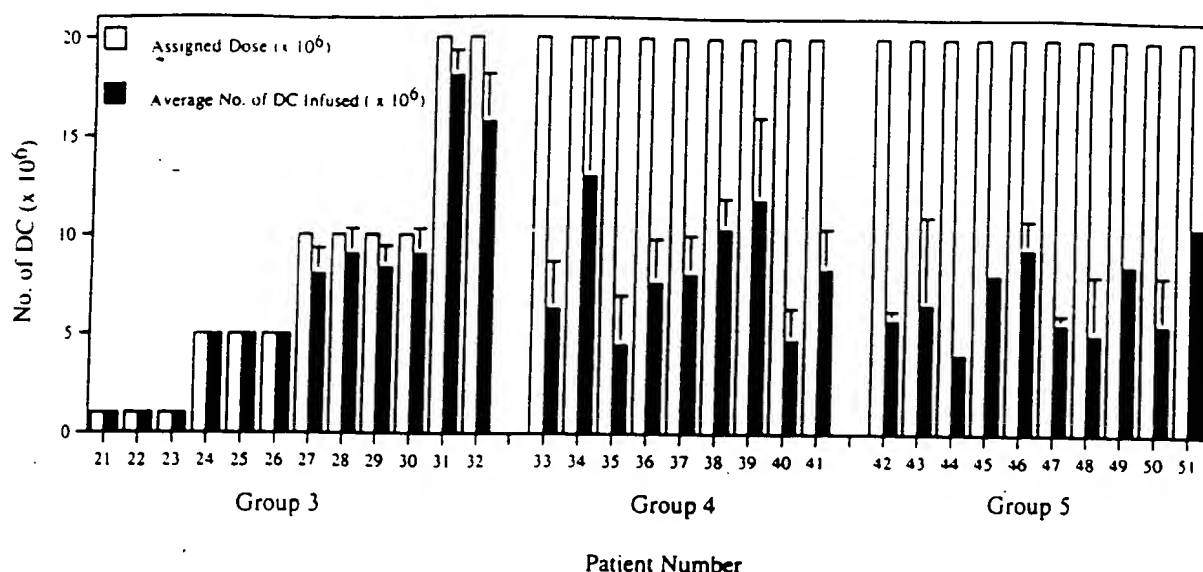


Fig. 1. Number of dendritic cells (DC) infused in this study. Three groups (groups 3–5) were infused with autologous DC cultured from individual patients' peripheral blood mononuclear cell population. Group 3 patients were divided into subgroups which received the assigned dose of 1, 5, 10, or  $20 \times 10^6$  DC. Groups 4 and 5 received the assigned dose of up to  $2 \times 10^7$  DC.

The actual number of cells infused depending on the number of DC harvested from culture before each infusions. The assigned dose, and the average number of DC actually infused (and standard error of the mean (SEM)) for each patient are depicted using white and black bars respectively. The average number of cell infused in groups 4 and 5 are  $8.2 \times 10^6$  and  $7.3 \times 10^6$ , respectively.

TABLE I. Phase I Patient Prostate Cancer Staging\*

Group	No. of patients	Stage				
		B2*	C*	D0*	D1*	D2
1	11	0	0	1	1	9
2	9	0	2	0	0	7
3	12	0	1	0	1	10
4	9	1	0	0	1	7
5	10	0	0	3	1	6
All	51	1	3	4	4	39

\*Patients in these stages also had elevations in PSA serum levels.

The second component was the requirement of efficient presentation of cancer Ag by the host's antigen presenting cells (APC) to circulating T cells in the generation of an effective anticancer response [17]. Dendritic cells (DC) are known as professional APC and have an exceptional ability to present Ag to naive CD4<sup>+</sup> and CD8<sup>+</sup> T cells both in vivo and in vitro [18,19]. DC are present in very small numbers in many tissues of lymphoid and nonlymphoid origin [19]. Several investigators, including our laboratory, have developed protocols to generate large numbers of DC from peripheral blood or bone marrow [20–24]. We further reported the ability to culture DC from peripheral blood of advanced

prostate cancer patients, many of whom with impaired immune system due to previous radiation, chemo-, or hormone therapy [20]. DC cultured from these patients pulsed with a number of prostate-specific Ag, including two HLA-A0201-specific PSMA peptides (designated PSM-P1 and PSM-P2), have the capacity to activate naive autologous T cells in vitro [12].

Based on the in vitro study, we conducted a phase I clinical trial to assess the safe administration of autologous DC pulsed with HLA-A0201-specific PSMA peptides to patients with advanced metastatic, hormone resistant prostate cancer. Patients were divided into five treatment arms that receive four or five infusions of PSM-P1 or PSM-P2 alone, autologous DC alone, or autologous DC pulsed with PSM-P1 or PSM-P2. Findings from this study support the potentiality of DC-based prostate cancer vaccines.

## MATERIALS AND METHODS

### Reagents and Cytokines

PSMA peptides with HLA-A0201-specific motif (PSM-P1: LLHETDSAV; PSM-P2: ALFDIESKV) were synthesized and purified (>95% purity) by Peninsula Laboratories (Belmont, CA) and obtained as a lyophilized powder. The powder was dissolved in 0.9% saline (USP 0.9%, sodium chloride injection, Ameri-

TABLE II. Clinical Profiles of Phase I Clinical Trial Participants\*

Group	No. of Patients	Hormone refractory	Postprostatectomy	Postradiation therapy	Postorchiectomy	HLA-A2+	(+ ) Skin tests			
							0	1	2	3
1	11	11	5	8	5	7	2	5	1	3
2	9	9	2	3	6	5	4	1	2	2
3	12	12	2	2	6	10	3	4	2	3
4	9	9	3	7	5	3	3	4	1	1
5	10	10	4	8	4	5	2	4	1	3
All	51	51	16	28	26	30	14	18	7	12

\*HLA-A2 expression was determined in our laboratory as described under Materials and Methods. Three types of skin test were conducted: diphtheria/tetanus, streptokinase, and mumps antigens.

TABLE III. Toxicity Observations in Phase I Study: Occurrences of Mild Hypotension\* Observed During Infusions

Infusion No.	Group 1	Group 2	Group 3	Group 4	Group 5	Total
1	5	2	5	4	3	19
2	0	1	1	1	0	3
3	4	1	3	1	0	9
4	1	0	1	0	ND*	2

\*Average systolic/diastolic over 4 hr <30/20 mm Hg (e.g., 136/89-116/69). All blood pressure decreases returned to pretreatment levels.

\*Data not available at the time of this analysis.

can Reagent Laboratories, Shirley, NY) to a concentration of 2 mg/ml. The peptide solution was sterilized using a 0.2- $\mu$ m filtration. Sterile 0.9% saline was added to achieve concentrations of 200, 20, and 2  $\mu$ g/ml; 100- $\mu$ l aliquots of the three peptide concentrations containing 20, 2, or 0.2  $\mu$ g peptide were transferred into sterile containers and frozen until use. Granulocyte-macrophage colony-stimulating factor (GM-CSF) approved for human use was provided by Immunex (Seattle, WA). Interleukin-4 (IL-4) approved for human use was provided by Schering-Plough Research Institute (Kenilworth, NY).

#### DC Culture

In this investigation, 50-100 ml peripheral blood was drawn and peripheral blood mononuclear cells (PBMC) isolated using Lymphoprep (GIBCO-BRL, Gaithersburg, MD) density-gradient centrifugation. PBMC were resuspended in complete medium (OPTIMEM medium [GIBCO-BRL, Gaithersburg, MD] and 5% heat-inactivated autologous plasma) and plated in a 75-cm<sup>2</sup> tissue culture flask ( $2-3 \times 10^7$  cells/flask). Cell suspensions were incubated in a humid-

ified incubator (37°C, 5% CO<sub>2</sub>) for 60 min. Non-adherent cells were removed and adherent cells were washed gently with warm (37°C) complete medium. Dendritic cell propagation medium (DCPM: complete medium, 1,000 U/ml GM-CSF and 1,000 U/ml IL-4) was added to the adherent cells (10 ml/flask). These cells were cultured for 4-6 days.

#### Patient Population

Fifty-one patients with advanced hormone-resistant prostate cancer were selected for the study, which included a signed informed consent. Their prostate cancer staging, previous treatment history, and pre-study delayed-type hypersensitivity (DTH) skin test results are shown in Tables I and II. In addition, patients were tested for expression of HLA-A2 expression using flow cytometric analysis with a monoclonal antibody specific for HLA-A2 (BB7.2 [25]), as well as polymerase chain reaction (PCR) (Table II). During the period of observation of 6-12 months, 50-100 ml of heparinized peripheral blood was drawn for DC culture and immunological monitoring.

#### Treatment Groups

Participants were divided into five treatment groups. In the first group, the patients were infused with PSM-P1 (LLHETDSAV) peptide at 0.2, 2.0, and 20.0  $\mu$ g; group 2 received PSM-P2 peptide (ALFDI-ESKV) at the same concentrations received by group 1; group 3 received autologous DC at 1, 5, 10, or  $20 \times 10^6$  per infusion; group 4 and 5 received up to  $2 \times 10^7$  autologous DC pulsed for 2 hr with 1  $\mu$ g/ml PSM-P1 or PSM-P2, respectively. Every group received four or five doses of the test substance at 6-8 week intervals during the study period.

#### Infusions

**PSMA peptides.** One frozen aliquot of PSM-P1 or -P2 containing the appropriate amount of peptide

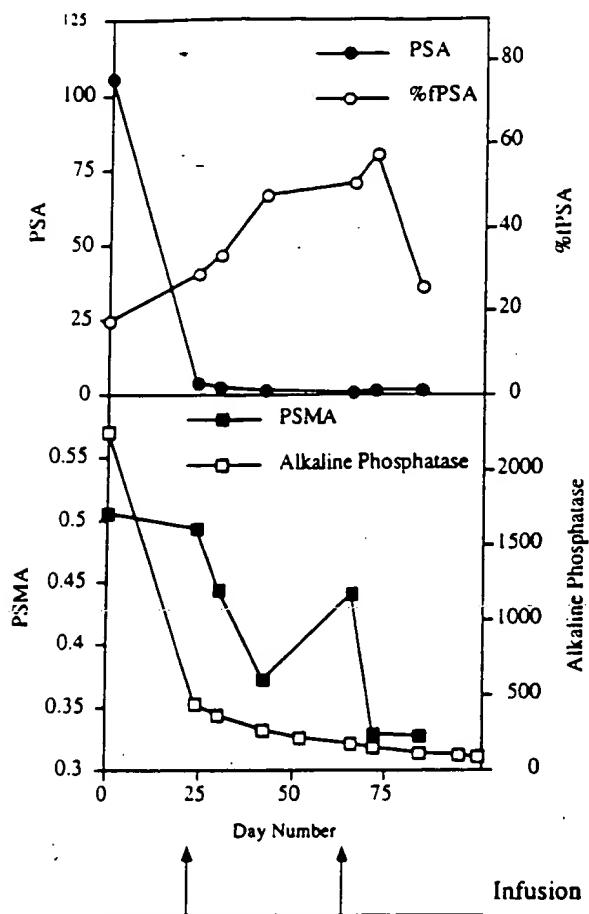


Fig. 2. Partial responder who had external radiation before vaccine therapy. This patient was admitted to this study with a history of bony metastasis (stage D<sub>2</sub>) and underwent palliative radiation therapy to his right shoulder and lower spine 3 weeks before the first infusion. He is an HLA-A2 positive individual enrolled in group 5. He was infused with 5 and 6 million autologous DC pulsed with PSM-P2 in the first two infusion cycles. The T-cell response to PSM-P2 was modulated after infusions (stimulation ratios postinfusion 1 = 1.9 and postinfusion 2 = 4.7). Top: Decrease in PSA and increase in % free PSA levels. Bottom: Decrease in PSMA and alkaline phosphatase levels during the course of the clinical trial. Arrows along the horizontal axis indicate the date of DC infusions.

(20, 2, or 0.2  $\mu$ g) was thawed and transferred into single-use 10-ml injectable USP 0.9% saline vials (American Reagent Laboratories). The peptide solution was infused over 5 min into the lateral entry port of a fast-flowing intravenous infusion of 0.9% saline.

**DC/Peptide-Pulsed DC.** Cells were washed three times with 0.9% saline to eliminate trace medium, counted, and transferred to into a single-use 10-ml injectable 0.9% saline. DC were infused over 30 min with a total volume of 100 ml 0.9% saline.

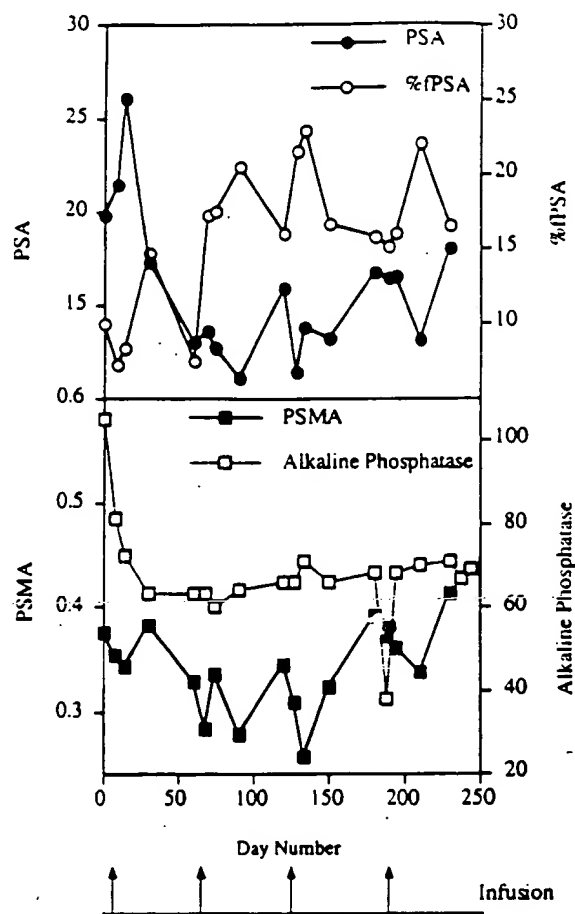


Fig. 3. Partial responder from group I who was in remission for 25–225 Days, and is now in progression.

### Toxicity Monitoring

Both acute and chronic toxicity were monitored. Monitoring for acute toxicity took place during and immediately following infusion for a period of 1–4 hr. Patients were observed for the development of (1) allergic reaction and (2) anaphylactic reaction during that time. Chronic toxicity were evaluated at periodic examinations.

### Cytokine Level Monitoring

Serum tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\alpha$ ) from peripheral blood drawn one day post-infusion were measured using enzyme-linked immunoabsorbent assay (ELISA) kits (BioSource International, Camarillo, CA).

### Clinical Monitoring

Patients were followed before during and after treatment with periodic PSA (prostate specific antigen; Tandem-E PSA kit, Hybritech, La Jolla, CA), free



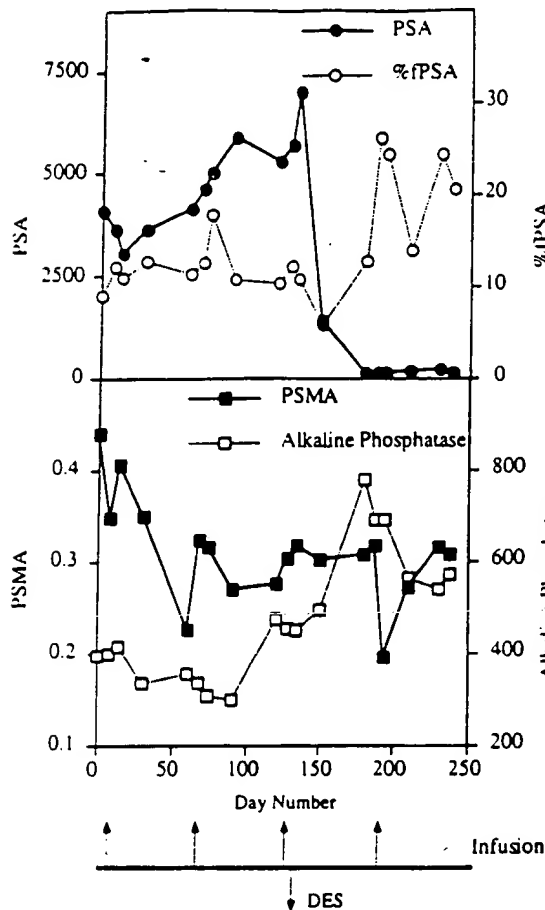


Fig. 4. Partial responder from group 2 who, at the time of the response, had a change in hormone therapy to DES.

PSA (Tandem-R PSA kit, Hybritech), PSMA, complete blood counts (CBCs), CHEM-22, initial chest radiography, bone scan, and computed tomography (CT) scan of the pelvis. All testing was conducted on either an inpatient or outpatient basis at Northwest Hospital. Patients were also evaluated every infusion cycle by one of the study physicians.

#### Immunological Monitoring

T-cell lymphoproliferative responses upon presentation of PSM-P1 or -P2 to patients' peripheral blood lymphocytes. In short, preinfusion and 7-day postinfusion PBMC freeze store vials were thawed, washed, cells counted, and viability assessed and resuspended at  $2 \times 10^6$  cells/ml; 200,000 cells per well were incubated in 96-well microtiter plates in triplicate. PSM-P1 or -P2 (10  $\mu$ g/ml) were added at the start of the culture. On day 5, 1  $\mu$ Ci tritiated thymidine ( $^3$ H-TdR) were added to each well. Cells were further incubated for 24 hr and harvested, and the tritiated thymidine incorporated was measured using a liquid

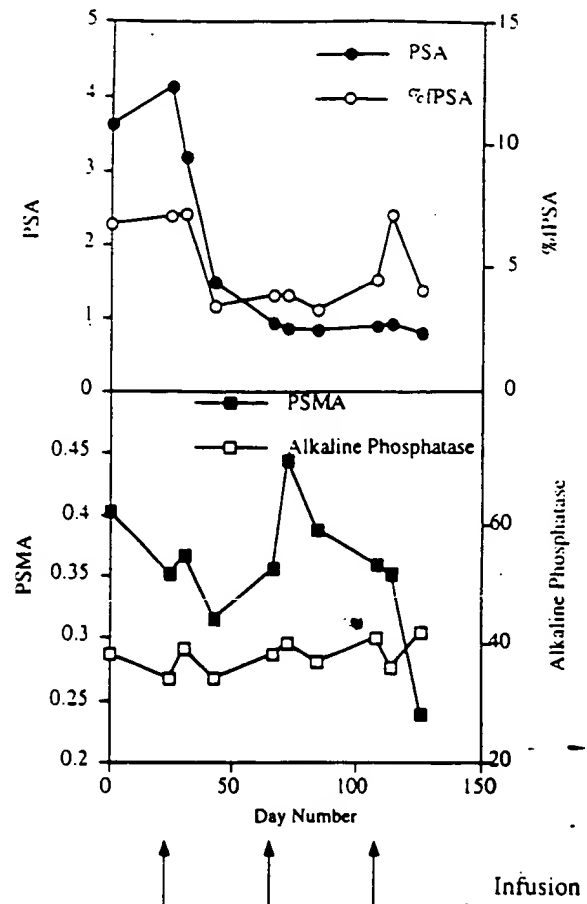


Fig. 5. Partial responder from group 4 in whom the % free PSA remains low at present.

scintillation counter. Stimulation ratio was calculated as the average cpm from postinfusion wells divided by the average cpm from preinfusion wells.

## RESULTS

### Patient Population

Fifty-one patients with hormone-refractory prostate cancer were selected for this study (Tables I, II). Most (47/51) have metastatic disease, most of whom (39/47) are stage D2 patients. A large proportion of the population have undergone previous radiation therapy (28/51) and/or surgical procedures, such as prostatectomy (16/51), orchiectomy (26/51), or a combination of treatments. Most study participants have impaired cellular immunity, as less than 25% (12/51) of the population responded to all three antigens in a DTH test conducted prior to the start of the study (Table II). More than 50% (30/51) of the patients in this study express HLA-A2 (Table II).

Study participants were divided into five treat-



ment groups as described under Materials and Methods. In short, patients in the first group received PSM-P1 at 0.2, 2.0, and 20.0  $\mu\text{g}$ ; group 2 received PSM-P2 at the same concentrations as group 1; group 3 received autologous DC at 1, 5, 10, or 20  $\times 10^6$  per infusion; and groups 4 and 5 received up to 2  $\times 10^7$  DC pulsed with PSM-P1 or -P2, respectively. The number of DC infused in groups 4 and 5 varied between 2  $\times 10^6$  to 2  $\times 10^7$  cells, depending on the number of cells cultured from patients' blood. Figure 1 shows the average number of DC infused to individual patients in groups 3–5. Patients in groups 1–3 have completed at least four infusions. Patients in groups 4 and 5 have completed at least three and two infusions, respectively.

### Toxicity Monitoring

Acute toxicity monitored during and immediately following infusion include the development of allergic and anaphylactic reactions. The only significant reaction observed was that of a mild to moderate hypotension (without associated pulse rate change, following the infusion in all groups (24/51 subjects). The incidence was highest at the first infusion (Table III) and declined thereafter. A given subject experiencing hypotension at the first infusion did not experience repeat hypotension on follow-up infusions in most cases (16/51 subjects did not experience repeat hypotension; 8/51 did, with 7 subjects experiencing hypotension in 2/4 infusions, and one subject experiencing hypotension in 3/4 infusions). Blood pressure reductions did not persist beyond 4 hr postinfusion. All subjects were recumbent during this observation period. No sequelae were noted. In addition, 3/51 patients reported a feeling of fatigue postinfusion that lasted 7–8 days. No significant rise in serum TNF- $\alpha$  or IFN- $\gamma$  was observed.

### Clinical and Immunological Monitoring

CBC and SMA-12 studies, as well as prostate marker studies were conducted at every interval. In addition, patients were also monitored for cellular immune modulation to the appropriate PSMA peptides (PSM-P1 or -P2). The results of these studies are summarized in Tables IV and V. Owing to the large variation of pretreatment levels of PSA among the patients, each treatment group was divided into two categories: low (initial PSA values of 0–10) and high (initial PSA values >10) PSA subgroups. Analysis of the five treatment groups as a whole demonstrated a modest increase in PSA levels in group 1–3 patients, who received peptide or DC alone, and group 4 patients, who received DC pulsed with PSM-P1, with high initial PSA. No significant change in PSA levels

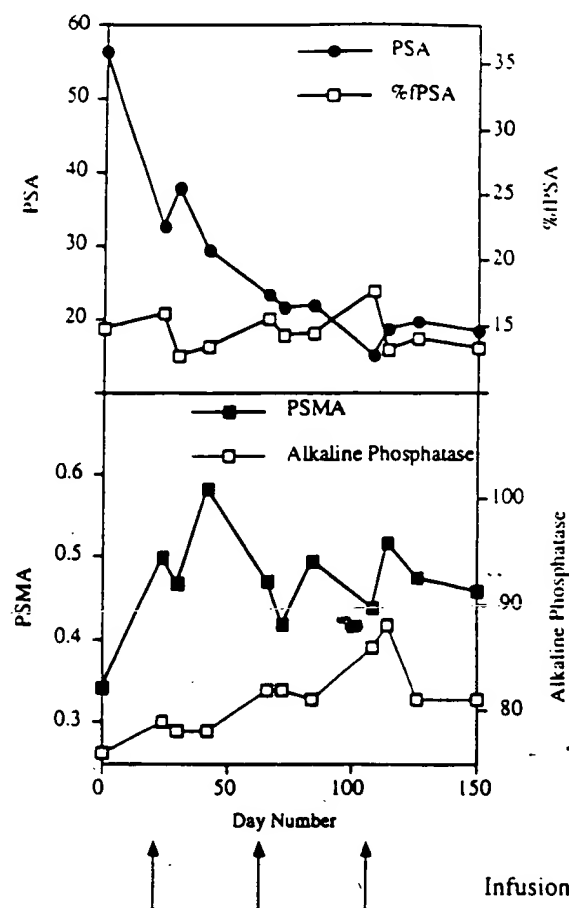


Fig. 6. Partial responder from group 4 in whom the PSMA values remain high.

was observed in group 4 patients with low initial PSA, as well as patients in group 5 (who received DC pulsed with PSM-P2; Table IV). In addition, no significant change was observed in the levels of absolute lymphocyte, hematocrit and alkaline phosphatase (Table V).

Analysis of cellular immune modulation as analyzed using an *in vitro* proliferation assay within each treatment group as a whole showed no significant response in groups 1–4 and a modest increase in group 5. However, a higher extent of cellular response are observed within the HLA-A2-positive subpopulation of groups 4 and 5 (Table VI), with an average stimulation ratio of 1.9 and 2.8 after two infusions of DC pulsed with PSM-P1 or -P2, respectively. Analyses of HLA-A2-positive populations within groups 1–2 (infused with peptide alone) or group 3 (DC alone) showed no significant increase in stimulation ratio. Furthermore, analyses of prostate markers within the HLA-A2-positive populations showed a decrease in PSA and a modest increase in

TABLE IV. Summary of Prostate Marker Levels\*

Group*	Subgroup	Category	Preinfusion		Postinfusion 1		Postinfusion 2		Postinfusion 3	
			Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1 PSM-P1	Low	PSA	1.71	0.76	1.81	0.76	2.72	1.19	8.63	4.87
		%fPSA	10.8	5.6	16.3	8.5	15.9	8.6	10.9	6
		[5] PSMA	0.24	0.04	0.24	0.05	0.3	0.03	0.34	0.05
	High	PSA	3.23	1.20	3.99	1.56	1.09	0.46	1.42	0.41
		%fPSA	18.4	7.2	15.4	4.7	21.2	1.7	18.1	2.5
		[6] PSMA	0.25	0.06	0.29	0.04	0.28	0.02	0.28	0.03
2 PSM-P2	Low	PSA	2.77	1.5	4.15	2.41	2.78	1.81	4.96	3.49
		%fPSA	5.6	3.6	4.3	2.3	6.3	3.5	6	2.7
		[5] PSMA	0.33	0.04	0.4	0.04	0.36	0.02	0.38	0.04
	High	PSA	1,099	994	862	728	1,369	1,211	2,891	1,812
		%fPSA	22	5.8	19.6	6.1	22.7	6.2	17.1	6
		[4] PSMA	0.27	0.09	0.25	0.08	0.26	0.05	0.32	0.02
3 Autologous DC	Low	PSA	7.24	1.81	8.41	3.2	11.4	5.66	10.5	4.16
		%fPSA	10.3	4.3	14.4	7.7	13.7	6	11.8	4.6
		[5] PSMA	0.29	0.02	0.29	0.04	0.29	0.04	0.28	0.01
	High	PSA	128	43	196	58	462	163	580	241
		%fPSA	27.8	6.4	23.6	6	21.1	5	24.3	6.5
		[7] PSMA	0.33	0.04	0.29	0.04	0.38	0.04	0.4	0.05
4 DC/PSM-P1	Low	PSA	2.39	0.93	1.91	0.91	1.27	0.63	2.08	1.2
		%fPSA	13.5	8.5	15.1	8.8	17.7	11.1	15.8	7.7
		[5] PSMA	0.37	0.04	0.39	0.05	0.39	0.03	0.34	0.02
	High	PSA	89	24	148	0.14	213	110	211	100
		%fPSA	16.4	2	13.9	0.5	12.4	0.7	15.2	0.9
		[4] PSMA	0.31	0.02	0.33	0.05	0.38	0.06	0.39	0.06
5 DC/PSM-P2	Low	PSA	3.34	0.83	2.6	0.3	3.5	0.47	—	—
		%fPSA	10.9	4	14.2	4.8	14.1	0.2	—	—
		[5] PSMA	0.35	0.05	0.36	0.05	0.35	0.04	—	—
	High	PSA	57.4	20.5	33	18	50.8	33.9	—	—
		%fPSA	15.2	3.6	19.2	5.1	26.2	9.9	—	—
		[4] PSMA	0.38	0.04	0.34	0.04	0.31	0.03	—	—

\*Each group is divided into low (initial PSA values 0–10) and high PSA (initial PSA values >10) categories. Normal ranges for the prostate markers: PSA = (0–4 ng/ml); %fPSA = (17.86 ± 1.89); PSMA = (0.08–0.21) (ref. 26). Mean and standard error of the mean (SEM) values are obtained from patient sera obtained preinfusion and 7 days postinfusion.

\*The number of patients in each subgroup is shown in parentheses.

percentage free PSA (%fPSA) levels only within patients in group 5 (Table VI).

### Responders

Individual analyses of patients' clinical response based on National Prostate Cancer Project (NPCP) criteria + PSA are presented in Table VII. Figure 2 reports the prostate markers and alkaline phosphatase level of one of the partial responders. This patient was admitted to this study with a history of bony metastasis (stage D<sub>2</sub>) and underwent palliative radiation therapy to his right shoulder and lower LS

spine 3 weeks before the first infusion. He is an HLA-A2 positive individual enrolled in group 5 (DC pulsed with PSM-P2 infusion). Immunological monitoring conducted showed increase in T-cell response to PSM-P2 peptide with each infusion (stimulation ratios postinfusion 1 = 1.9 and postinfusion 2 = 4.7) (Fig. 2). His PSA level decreased from an initial value of 105.51 to 3.54 after the first infusion cycle and stayed stable at 0.53–1.08 over 11 weeks of observation. In addition, decreasing levels of PSMA (from 0.506 to 0.326) and alkaline phosphatase (from 2,245 to 116), and an increase of %fPSA (17.2 to 57.4) were observed. Furthermore, a bone scan conducted after

TABLE V. Clinical Monitoring Summary\*

Group <sup>a</sup>	Category	Preinfusion		Postinfusion 1		Postinfusion 2		Postinfusion 3		Postinfusion 4	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	Stimulation ratio <sup>b</sup>	—	—	1.09	0.03	1.59	0.3	1.33	0.17	—	—
[11]	Absolute lymphocyte	852	159	690	122	852	85	729	105	509	53
	Hematocrit	35.6	1.2	34.9	1.5	35.8	1.9	35.6	1.7	33	1.5
	Alkaline phosphatase	152	39.8	184	60	136	31	145	36	140	23
2	Stimulation ratio <sup>b</sup>	—	—	1.22	0.17	1.32	0.13	1.26	0.2	—	—
[9]	Absolute lymphocyte <sup>c</sup>	1,440	340	1,296	283	1,298	330	1,189	26	1,660	268
	Hematocrit <sup>d</sup>	36.6	1.8	35	1.7	35.8	2.3	34.5	2	36.2	2.1
	Alkaline phosphatase <sup>e</sup>	127	37	126	37	112	26	144	44	137	48
3	Stimulation ratio <sup>b</sup>	—	—	1.13	0.06	1.2	0.06	1.23	0.07	—	—
[12]	Absolute lymphocyte <sup>c</sup>	1,322	82	1,341	134	1,292	103	1,345	129	—	—
	Hematocrit <sup>d</sup>	38.6	1.9	37.6	1.9	36.5	2.1	37.6	2.2	—	—
	Alkaline phosphatase <sup>e</sup>	145	30	179	51	293	104	371	160	—	—
4	Stimulation ratio <sup>b</sup>	—	—	1.87	0.34	1.37	0.21	1.2	0.12	—	—
[9]	Absolute lymphocyte <sup>c</sup>	833	144	838	129	800	76	1,037	143	—	—
	Hematocrit <sup>d</sup>	37	0.9	36.9	0.8	37	0.5	38.3	0.6	—	—
	Alkaline phosphatase <sup>e</sup>	67	9	69	11	73	11	79	16	—	—
5	Stimulation ratio <sup>b</sup>	—	—	1.33	0.07	2.01	0.42	—	—	—	—
[10]	Absolute lymphocyte <sup>c</sup>	1,155	202	1,026	178	972	162	—	—	—	—
	Hematocrit <sup>d</sup>	37.8	1.5	37.8	1.1	37.4	1	—	—	—	—
	Alkaline phosphatase <sup>e</sup>	323	225	111	146	103	12.5	—	—	—	—

\*Mean and standard error of the mean (SEM) values are obtained from patient sera obtained preinfusion and 7 days postinfusion.

<sup>a</sup>The number of patients in each group is shown in parentheses.

<sup>b</sup>Measure of T-cell lymphoproliferative response against the appropriate PSMA peptide as described under Materials and Methods.

<sup>c</sup>Normal range: 1,000–3,500 × 10<sup>3</sup> ml.

<sup>d</sup>Normal range: 40–52%.

<sup>e</sup>Normal range: 25–125 IU/L.

two infusion cycles revealed a considerable improvement with decreased activity seen throughout the skeletal metastasis compared to two previous examinations conducted before his enrollment in the study and after one infusion cycle.

Figure 3 illustrates a partial responder from group 1. The response lasted 25–225 days and was of short duration. A bone scan has confirmed the progression. Figure 4 shows a partial responder from group 2. The hormone treatment was changed to 1 mgm/qd D.E.S. Thus, the origin of the response is not clear. As shown in Figure 5, a responder in group 4 did not show a resolution of all four markers (PSA, %fPSA, alkaline phosphatase, and PSMA). The %fPSA was not restored to normal ranges, and the normal alkaline phosphatase remained such. By contrast, as shown in Figure 6, another group 4 responder had no response in terms of the PSMA levels. For the determination of a response, the NPCP criteria plus PSA reduction >50% was required. The additional mark-

ers are under comparative study. Thus, to date, seven partial responders have been identified along with 29 progression, 11 stable, and 4 nonevaluable cases.

## DISCUSSION

In this study, we have examined the safe administration of HLA-A0201-specific PSMA peptides (PSM-P1 and -P2), autologous DC, and PSM-P1 and -P2 pulsed autologous DC to 51 patients with advanced hormone-refractory prostate cancer, most of whom (39/51) are in stage D<sub>2</sub>. Many of these patients are anemic and have undergone various treatments that have resulted in an impaired immune competency. Less than 25% of this population were considered fully immunocompetent at the start of the study, as assessed by DTH skin tests. Almost one-third of these patients failed to respond to any of the three test antigens.

At the completion of up to four cycles of infusion,

TABLE VI. PSA Levels and T-Cell Response Against PSMA Peptide in HLA-A2-Positive Patients\*

Group*	Category	Preinfusion		Postinfusion 1		Postinfusion 2		Postinfusion 3	
		Ave	SEM	Ave	SEM	Ave	SEM	Ave	SEM
1	Stimulation ratio*	—	—	1.01	0.1	1.39	0.31	1.16	0.18
	PSA	4.47	3.17	5.53	4.23	4.01	2.03	9.24	4.18
[7]	% freePSA	10.5	5.17	16.1	7.62	18.9	7.29	13.6	5.6
2	Stimulation ratio*	—	—	1	0.07	1.1	0.05	1.3	0.25
	PSA	875	896	684	661	1086	1095	2146	1.656
[5]	% freePSA	17.3	7.55	15.8	7.48	19.6	7.7	14.3	6.8
4	Stimulation ratio*	—	—	2.2	0.29	1.9	0.21	—	—
	PSA	74.1	38.9	93.3	51.3	177.7	132	211.6	145.8
[3]	% freePSA	26	10.5	26.5	11	30.9	14.2	29.6	10.4
5	Stimulation ratio*	—	—	1.6	0.07	2.8	0.43	—	—
	PSA	31.8	21.1	10.35	5.28	12.1	6.23	—	—
[6]	% freePSA	15.8	4.46	20.7	5.87	23	11.8	—	—

\*The number of HLA-A2-positive patients in each group is shown in parentheses.

\*Measure of T-cell lymphoproliferative response against the appropriate PSMA peptide depicting the ratio of postinfusion and preinfusion values.

TABLE VII. Summary of Phase I Trial Clinical Status\*

Group	Pro- gression	Stable	Partial responder	Non- evaluable	Total	HLA-A2 +/-
1	7	3	1	0	11	7/4
2	5	3	1	0	9	5/4
3	10	2	0	0	12	11/12
4	3	2	4	0	9	3/6
5	7	1	1	1*	10	6/3

\*Clinical status was determined as of 9/16/96 using NPCP criteria + PSA.

\*One patient died before receiving any infusion.

maximum tolerated dose was not achieved, as no significant acute or chronic toxicity was observed in all doses of test substances. Elevation of the level of serum TNF- $\alpha$  and IFN- $\gamma$ , which are usually associated with immune response-mediated toxicity, were absent in all study groups [27]. The only effect observed were mild to moderate cases of hypotension, presumably due to the length of infusion and observation period immediately following infusion, in which patients were recumbent. Blood pressure drop did not persist over 4 hr postinfusion.

Data from immunological monitoring studies show an increase of T-cell response to the appropriate PSMA peptides in HLA-A2-positive patients in groups 4 and 5. These patients received infusions of autologous DC pulsed with PSM-P1 or -P2, respectively, implying specific in vivo presentation of the PSMA peptides

with HLA-A0201-specific motif, by autologous DC to circulating T cells. No significant responses were observed in group 3 patients, who received autologous DC alone, as well as group 1 and 2 patients, who received peptides alone. These results demonstrate the requirement of both components in the generation of an effective immune response.

PSA assay is the only Food and Drug Administration (FDA)-approved clinical assay for monitoring and early detection of prostate cancer to date. HLA-A2-positive patients in group 5 exhibited a significant reduction in average PSA values, presumably due to lysis of prostate cancer cells by PSM-P2-specific autologous T cells. Based on the NPCP criteria + PSA, seven partial responders were identified. Only one of the partial responders was identified from group 5 after two cycles of infusions. Four non-evaluable patients are all from group 5, two of whom are HLA-A2 positive and will continue to receive infusions and clinical evaluations for the rest of the study period.

The seven responders have exhibited responses of 150–225 days duration to date. These are relatively short. In five of these seven patients, all four markers were changed, viz. total PSA, % free PSA, PSMA, and total alkaline phosphatase. These are being further studied. We will soon also test the direct measurement of bone alkaline phosphatase isozymes. In follow-up evaluation, additional responses may be identified. At present we feel justified in planning a phase II study which will include DC pulsed with PSMA or PSMA peptides.

## ACKNOWLEDGMENTS

We appreciate the assistance of Sheila Erickson and Victoria Bowes and the encouragement of S. Leslie Misrock. This work was supported by a grant from the CaPCURE Foundation and Phi Beta Psi Sorority.

## REFERENCES

- Traversari C, van der Bruggen P, Luescher IF, Lurquin C, Chomez P, Van Pel A, De Plaen E, Amar-Costesec A, Boon T: A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. *J Exp Med* 176:1453-1457, 1992.
- Peoples GE, Goedegebuure PS, Smith R, Linehan DC, Yoshino I, Eberlein TJ: Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER2/neu-derived peptide. *Proc Natl Acad Sci USA* 92:432-436, 1995.
- Ioannides CG, Fisk B, Pollack M, Frazier ML, Wharton JT, Freedman RS: Cytotoxic T cell clones isolated from ovarian tumor-infiltrating lymphocytes recognize common determinants on non-ovarian tumour clones. *J Immunol* 150:413-424, 1993.
- Urban JL, Schreiber H: Tumor antigens. *Annu Rev Immunol* 10:617-644, 1992.
- VanderBruggen P, Traversari C, Chomez P, Lurquin C, DePlaen E, VanderEynde B, Knuth A, Boon T: A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 254:1643-1647, 1991.
- Zinkernagel RM, Doherty PC: Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature* 248:701, 1974.
- Engelhard VH: Structure of peptides associated with class I and class II MHC molecules. *Annu Rev Immunol* 12:181-207, 1994.
- Falk K, Rotzchke O, Stevanovic S, Jung G, Rammensee HG: Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* 351:290-296, 1991.
- Grey HM, Ruppert J, Vitiello A, Sidney J, Kast WM, Kubo RT, Sette A: Class I MHC-peptide interactions: Structural requirements and functional implications. *Cancer Surv* 22:37-49, 1995.
- Ruppert J, Sidney J, Celis E, Kubo R, Grey H, Sette A: Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules. *Cell* 74:929-937, 1993.
- Tsuji K, Aizawa M, Sasazuki T: Allele frequencies, Section 6.3: Splits combined (five loci). In "Proceedings of the Eleventh International Histocompatibility Workshop and Conference," Nov. 6-13: 1991, Yokohama, Japan. Tokyo: Oxford University Press, pp 1066-1077, 1992.
- Tjoa B, Boynton A, Kenny G, Ragde H, Misrock SL, Murphy G: Presentation of Prostate tumor antigens by dendritic cells stimulates T-cell proliferation and cytotoxicity. *Prostate* 28:65-59, 1996.
- Horoszewicz JS, Kawinski E, Murphy GP: Monoclonal antibodies to a new antigenic marker in epithelial prostate cells and serum of prostatic cancer patients. *Anticancer Res* 7:927-936, 1987.
- Israeli RS, Powell CT, Corr JG, Fair WR, Heston DW: Molecular cloning of a complementary DNA encoding a prostate-specific membrane antigen. *Cancer Res* 54:1807-1811, 1993.
- Murphy G, Ragde H, Kenny G, Barren R III, Erickson S, Tjoa B, Boynton A, Holmes E, Gilbauch J, Douglas T: Comparison of prostate specific membrane antigen, and prostate specific antigen levels in prostatic cancer patients. *Anticancer Res* 15:1473-1480, 1995.
- Murphy GP, Holmes EH, Boynton AL, Kenny GM, Ostenson RC, Erickson SJ, Barren RJ III: Comparison of prostate specific antigen, prostate specific membrane antigen, and LNCaP-based enzyme-linked immunoabsorbent assays in prostatic cancer patients. *Prostate* 26:164-168, 1995.
- Knight SC, Stagg AJ: Antigen-presenting cell types. *Curr Opin Immunol* 5:374-382, 1993.
- Grabbe S, Beissert S, Schwartz T, Granstein RD: Dendritic cells as initiators of tumor immune response: A possible strategy for immunotherapy? *Immunol Today* 16:117-121, 1995.
- Steinman RM: The Dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 9:271-296, 1991.
- Sallusto F, Lanzavecchia A: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 179:1109-1118, 1994.
- Tjoa B, Erickson S, Barren R III, Ragde H, Kenny G, Boynton A, Murphy G: In vitro propagated dendritic cells from prostate cancer patients as a component of prostate cancer immunotherapy. *Prostate* 27:63-39, 1995.
- Freudenthal PS, Steinman RM: The distinct surface of human blood dendritic cells, as observed after an improved isolation method. *Proc Natl Acad Sci USA* 87:7698, 1990.
- Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM, Schuler G: Proliferating dendritic cell progenitors in human blood. *J Exp Med* 180:83-93, 1994.
- Berhard H, Disis ML, Heimfeld S, Hand S, Graal w JR, Cheever MA: Generation of immunostimulatory dendritic cells from human CD34+ hematopoietic progenitor cells of the bone marrow and peripheral blood. *Cancer Res* 55:1099-1104, 1995.
- Parham P, Brodsky FM: Partial purification and some properties of BB7.2. A cytotoxic monoclonal antibody with specificity for HLA-A2 and a variant of HLA-A28. *Hum Immunol* 3:277-299, 1981.
- Murphy GP, Barren RJ, Erickson SJ, Bowes VA, Wolfert RL, Bartsch G, Klocker H, Pointner J, Reissigl A, McLeod DG, Douglas T, Morgan T, Kenny GM, Ragde H, Boynton AL, Holmes EH: Evaluation and comparison of two new prostate carcinoma markers: Free-prostate specific antigen and prostate specific membrane antigen. *Cancer* 78:809-818, 1996.
- Thom A, Alexander H, Andrich M, Barker W, Rosenberg S, Fraker D: Cytokine levels and systemic toxicity in patients undergoing isolated limb perfusion with high-dose tumor necrosis factor, interferon gamma, and melphalan. *J Clin Oncol* 13:264-273, 1995.